

Department Virology
Subject Recombinant VEE
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Address MIIB

Lab Notebook # 3492
National Brand 43-648
Issued 12-28-92

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701



0 73333 43648 8

75 Sheets
11 3/4" x 9 1/4"
4x4 Quad.

EXHIBIT R

MgCl₂ OPTIMIZATION

21 DEC 92

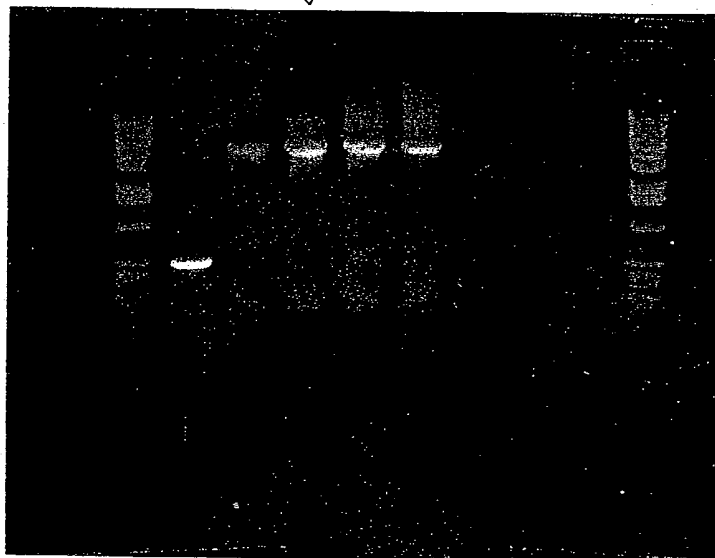
BEST AVAILABLE COPY

MgCl ₂ (mM)		1	1.5	2	3
DNA →	λC	20WAT			
		5			
FORWARD PR	Pr # 1	V26MF1			
REVERSE PR	Pr # 2	V26MR3			
EXPECTED LENGTH (bp)	500	~3900			
H ₂ O (μl)	70.5	74.5	72.5	70.5	66.5
NA TEMPLATE	1	0.5	0.5	0.5	0.5
FORWARD PR	5	1.3	1.3	1.3	1.3
REVERSE PR	5	1.2	1.2	1.2	1.2
10X RXN BUFFER	18	18	18	18	18
10mM dNTP					
5mM MgCl ₂	—	4	6	8	12
Topol	0.5				
	Vortex, microfuge				
Mineral oil	100 μl				

Thermocycler program
 Stop cycle: 30 sec @ 95°C
 35 cycles { 30 sec @ 52°C
 3 min @ 72°C
 Delay cycle: 10 min @ 72°C
 Soak cycle: 4°C

Master Mix (II)

10X Buffer 4 x 10 = 40
 dATP 2 x 6 = 12
 dGTP
 dCTP
 dTTP
 TV 10%



I chose the 1.5 mM of MgCl₂ to run the 5X amplification. It seemed to be the best lane. Hope your final went well. See you on Wednesday.

Cathy

Deletion Primers

ANCHOR begins at 3877

VDAM-RZ

general new primer - this one would produce an anchor

5' > G T A A A A C C A C C C C A C T A G C T T C T T C T A T

G T A A C C C A C C T T C C < 3' (Stop = 3793)

PCR: 100 pmol / rxn

Use 2 ul / rxn

M13/pUC7

5' > G T A A A A C C A C C C C A C C

PCR: Need 100 pmol / rxn

Use 0.12 ul / rxn

Tm C = 64°C

VD BFI

5' G A G G T G A T A A C T C A T T A T T A C C A C A C A C A C

A C C A C C T E G C A G < 3'

PCR: Need 100 pmol / rxn

Use 1.4 ul / rxn

Tm C = 91°C

1st round PCR

Template: ZOWAT 5' (0.5 ul)

Primers: V D B - F I 1.4 ul (100 pmol)

V D A M - R Z 2.0 ul (100 pmol)

Tag pol: 0.5 ul

2 min @ 95°
2 min @ 37°
3 min @ 72° } 30 cycles

VDAM-RZ 3830 + 23 (Read + M13 = 3853)

VD BFI 2092 - 2118 = 26

Deleted 2119 → 2301 (182 bp)

2302 → 3853 = 1551

1551

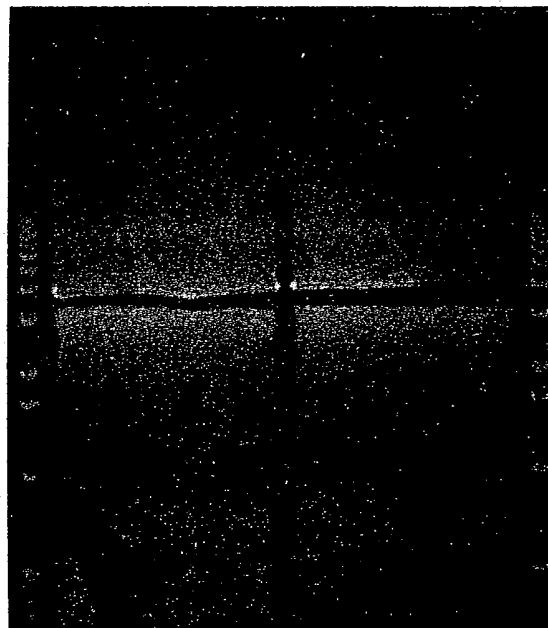
36

1576 bp

Deletion = 1576 bp

C.C. etc

A260/280 = 3.5485 ug / 50 ul



Column 1

11:10:28A, 12/27/92

Run ID :

Cycle : Cyc02 user

End Proc: End CESS (DMT = On)

Sequence: VAR3

Total bases = 35

A= 16, G= 5, C= 6, T= 8, S= 0, B= 0, 7= 0, 8= 0
(mixed bases= 0)

MW: 11664.0

5' > GGT ACT CAA AGC TTG AAT ATT AAA AAC AAA ATC CG <3'

$$\begin{aligned}
 E_m &= A(16,000) + C(7000) + G(12,000) + T(9600) \\
 &= 16(16,000) + 6(7000) + 5(12,000) + 8(9600) \\
 &= 256,000 + 42,000 + 60,000 + 76,800
 \end{aligned}$$

$$E_m = 434,800$$

$$\text{Molarity} = \frac{0.0260 \times \text{dil factor}}{E_m}$$

$$= \frac{0.1591 \times 101}{434,800}$$

$$= 16,0691$$

$$= 434,800$$

$$= 0.000036957$$

$$\text{Molarity} = 36.7 \text{ pmol/lit}$$

Need 100 pmol for PCR

Use 2.7 ul / rxn

DUAL	λ	01	Abs	Factor= 1.000	Read average = 01		
SW	260.0	280.0	λA/λB	SW	260.0	280.0	λA/λB
Sub in 500ul H ₂ O = 1:101 dil. 200ul total/sample							
1	0.0001	0.0001	1.000				
2	0.1591	0.0924	1.721	VAR3	29 DEC 92		
3	0.2631	0.1740	1.512	VE3R2			
4	0.2124	0.1421	1.494	VE2R2			

λ ABS SOURCE 11:41 CELL
260.0 0.0002 UV/Vis 12/30/92 1

1st ROUND PCR

29 DEC 92

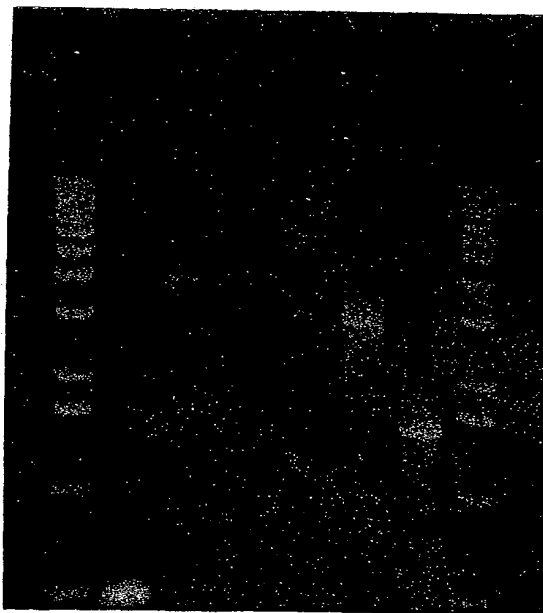
2

3

4

5

DNA →	7C	DNA PREP 20 WATS V26 MF1/V26 MR3 G-8, GC, 22 DEC 92				
		3914	3,095	3,095	2,800	1445
FORWARD PR.	Pr#1	VC-F1	VE3-F1	VE3-F2	VE3 F1	VE3 F1
REVERSE PR.	Pr#2	VA-R3	VAR3	VAR3	VE2 R2	VE3 R2
H ₂ O	10.5	76.6	76.3	76.2	76.8	77.3
DNA Template	1	1	1	1	1	1
FORWARD PR.	5	1.2	1.5	1.6	1.5	1.5
REVERSE PR.	5	2.7	2.7	2.7	2.2	1.7
10X RXN BUFFER	{ MME	18 ul	18	18	18	18
10mM dNTP						
	Vortex, microfuge					
Taq pol	0.5 ul	0.5	0.5	0.5	0.5	0.5
		22.4	23.7	23.2	23.2	22.7
TV		100	100	100	100	100
Minicolumn	100	100	100	100	100	100
Thermocycler Program:		Phastu Thyl I				
Step cycle: 30 sec @ 95°C		10X Rax Buffer, T = 7x 10 = 70 ul				
30 sec @ 52°C		d ATP 2x 7 = 14 ul				
3 min @ 72°C		d CTP " 14				
Delay 10 min @ 72°C		d GTP " 14				
Soak: 4°C		d TTP " 14				



Column 4

6:15:52P ,12/28

Run ID :

Cycle : Cyc02 user

End Proc: End CESS (DMT = On)

Sequence: VDMRI

WRONG SEQ

---- S I M P L Total bases = 44

A= 19, G= 9, C= 8, T= 8, 5= 0, 6= 0, 7= 0, 8= 0

(mixed bases= 0)

Sequence: 5'-

MW: 14695.8

Len 5' > 66C TTA GCC TAA AAC AAA AAT TAT AAG TGA CCG GCA GCA

M13

Com AAA TG <3'

%GC $E_m = A(16000) + C(7000) + G(12000) + T(9600)$

$= 19(16000) + 8(7000) + 9(12000) + 8(9600)$

Tm $= 304,000 + 56,000 + 108,000 + 76,800$

$E_m = 544,800$

Sec Molarity = $\frac{0.0260 \cdot \text{dil factor}}{E_m}$

Run $= \frac{0.3113 \cdot 101}{544,800}$

$= 0.0000577$

Molarity = 57.7 pmol

PCR: Need 100 pmol/μm

Uses 1.7ul/μm.

Not working

DUAL A 01

Abs

Factor= 1.000

Read average = 01

SH 260.0 280.0 NA/NB SH 260.0 280.0 NA/NB

But in 500 ul H₂O = 1:100 dil

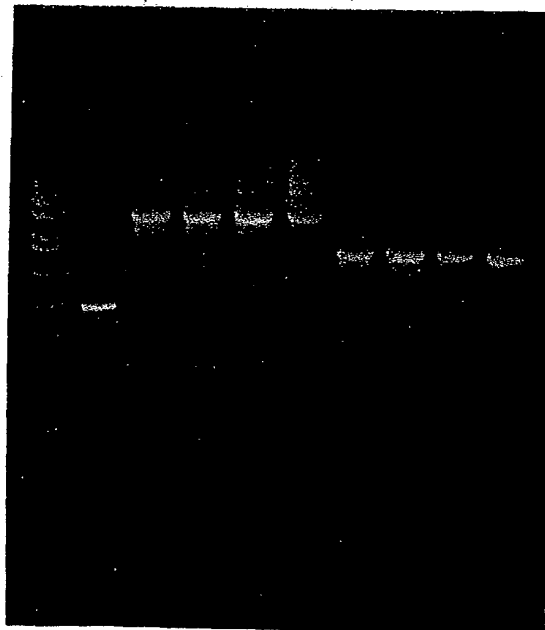
1	-0.0001	-0.0003	1.3086	
2	0.3113	0.1709	1.822	VDMRI 31DEC92
3	0.5497	0.3647	1.507	VDCR1 "
4	0.2806	0.1619	1.733	M13 "
5	0.3509	0.2223	1.578	VDB-F1

λ	ABS	SOURCE	09:54	CELL
260.0	-0.0002	UV/Vis	01/04/93	1

4JAN93

MgCl₂ OPTIMIZATION

EMgCl ₂ mM →		1	1.5	2	3	1	1.5	2	3
DNA	2C	20 WAT 5-V 26MFI/V26MR3 GB, GC, 22 DEC 92							
FORWARD PR	Pr#1	VE3F1	→				VE3F1	→	
VERSE PR	Pr#2	VE2R2	→				VE3R2	→	
ROUTED	500								
LENGTH (bp)									
DO	70.5	73.3 4.8	71.3 4.8	69.3 30.4	65.3 34.7	73.8 20.2	71.8 28.2	69.8 30.2	65.8 34.2
DATE/TEMPLATE	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
FORWARD PR	5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
VERSE PR	5	2.2	2.2	2.2	2.2	1.7	1.7	1.7	1.7
RXN BUFFER	10	18.0	18	18	18	18	18	18	18
NTP	2 ut ea								
mM MgCl ₂	—	4	6	8	12	4	6	8	12
pol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
mineral oil	100								
thermocycler program									
step cycle: 30 sec @ 95°C									
30 sec @ 52°C									
3 min @ 72°C									
delay cycle: 10 min @ 72°C									
soak cycle: 4°C									
Master Mix									
Buffer III 9x10 = 90 ul									
dATP 9x2 = 18									
dCTP 9x2 = 18									
dGTP									
dTTP									



12-384

5X Amplification

4 JAN 92

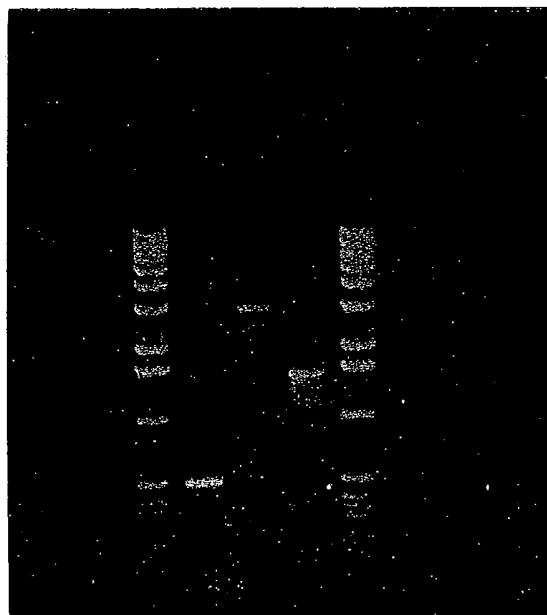
1X

[5X]

1X

[5X]

DNA	λC	ZOWAT	5-V26 MF1/V26 MR3	GB, GC, 22 DEC 92	
FORWARD PR	Pr#1	VE3F1	→	VE3F1	→
REVERSE PR	Pr#2	VE2R2	→	VE3R2	→
EXPECTED LENGTH (bp)	500	2,800		1,445	
H ₂ O	70.5	73.3	366.5	73.8	369
DNA TEMPLATE	1	0.5	2.5	0.5	2.5
FORWARD PR	5	1.5	7.5	1.5	7.5
REVERSE PR	5	2.2	11	1.7	8.5
10X RXN BUFFER	18	18μl (II)	90	18μl II	90
10mM dNTP	2μl each	—	—	—	—
25mM MgCl ₂	—	4	20	4	20
Taq pol	0.5	0.5	2.5	0.5	2.5
Thermal oil	100	—	—	—	—
Thermocycler program:					
Step cycle: 30sec @ 95°C					
30sec @ 52°C					
3min @ 72°C					
Delay cycle: 10min @ 72°C					
Soak cycle: 4°C					
Master mix (II)					
10X Rxn buffer: 12 x 10 = 120μl					
dATP: 12 x 2 = 24					
dCTP: " = 24					
dGTP: " = 24					
dTTP: " = 24					
216μl					



DUAL λ 01 Abs Factor= 1.000
Read average = 01
SW 260.0 280.0 $\lambda A/\lambda B$ SW 260.0 280.0 λA
5 ml in 500 ml H₂O
1 0.0001 0.0006 .2500
2 0.2399 0.1566 1.531 *VDAMR2*

λ ABS SOURCE 13:20 CELL
260.0 0.0007 UV/VIS 01/08/93 1

5 JAN 93



VE3

5 JAN 93



VE2

- 394 Synthesis Setup Listing - (Version 1.01)

Column 1

10:11:03A , 1/ 6/93

Run ID :

Cycle : Cyc02 user

End Proc: End CESS (DMT = On)

Sequence: VDAMR2

Total bases = 43

A= 12, G= 11, C= 9, T= 11, S= 0, B= 0, 7= 0, 8= 0

(mixed bases= 0)

MW: 14229.6

5' > GTA AAA CGA CGG CCA GTA AGC TTG TTC TAT GTA AGC AGC
 MIS 3830

TTG C <3'

$$\begin{aligned}
 E_m &= A(16000) + C(17000) + G(12000) + T(9600) \\
 &= 12(16000) + 9(17000) + 11(12000) + 11(9600) \\
 &= 192,000 + 153,000 + 132,000 + 105,600 \\
 E_m &= 492,600
 \end{aligned}$$

$$\text{Molarity} = \frac{0.0260 \cdot \text{dil factor}}{E_m}$$

$$= \frac{0.2399 \cdot 101}{492,600}$$

- A A A A A 491

12 JAN 93

ZOWATT 5-V26MF1/V26MR3 = DNA TEMPLATE

PRIMERS: VE3F1/VE2R2 = ~2,800 bp

VE3F1/VEBR2 = 1,495 bp

Bam HI, Hind III digestion

EB, GC

$[MgCl_2]_{mM} \rightarrow$ 1 1.5 2 3

DNA →	λC	ZOWAT 5				5X
FORWARD PR	Pr #1	V26MF1				
REVERSE PR	Pr #2	V26MR2				
EXPECTED LENGTH(bp)						
H ₂ O(μl)	70.5	74.9	72.9	70.9	66.9	364.5
DNA TEMPLATE	1	0.5	0.5	0.5	0.5	2.5
FORWARD PR	5	1.3	1.3	1.3	1.3	6.5
REVERSE PR	5	0.8	0.8	0.8	0.8	4.0
10X RXN BUFFER		18	18	18	18	90
10mM dNTP						
25mM MgCl ₂	—	4	4	8	12	30

TAQ	COL	Vortex, microfuge		
		0.5 ml	0.5	0.5

Mineral oil	100 cc
-------------	--------

Thermocycle Program:

Step cycle : 30 sec @ 950°C

30 sec. @ 52°C

3 min @	72°C
---------	------

display cycle: 10 min @ 72°C

Isak. 4°C

Master Mix (II)

Buffer	$5 \times 10 = 50$	ml
dATP	$5 \times 2 = 10$	
dCTP	"	

Buch	$6 \times 10 =$	60
------	-----------------	----

$$dA \approx 6 \times 2 = 12$$

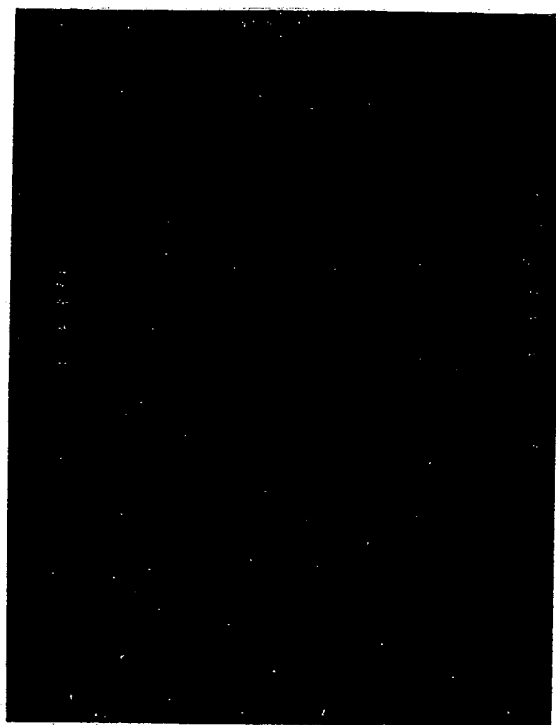
д.с.р.		
1980		

d GTP		
1 TTP		

d tip	
	TUT 105

TV 108

2004



101 ROUNDS FOR

8 JAN 93

1

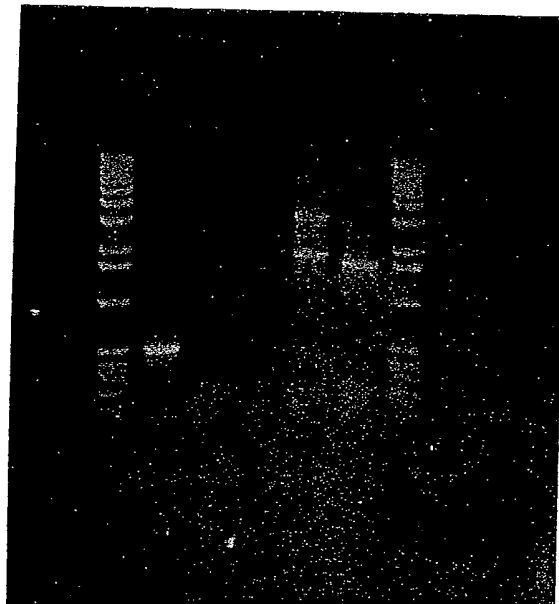
2

3

4

8 JAN 93
REPEAT 0.5 #1, 2 w/ ZOWAT

DNA →	λC	PCR PRODUCT	GB, GC	75 JAN 93	ZOWAT 5/V26 MF1/V26 MR2	ZOWAT 5	ZOWAT 5	C
FORWARD PR	P1#1	VC-F1	VE3-F1	VE3-F2	V6K-F1	VC F1	VE3 F1	Pr#1
REVERSE PR	P1#2	VA-R3	VA-R3	VAR3	VAR3	VAR3	VAR3	Pr#2
EXPECTED LENGTH (bp)	500	3,914	3,195	3,095	1,646	3,914	3,095	500
H ₂ O	20.5	76.6	76.3	76.2	76.7	77.1	76.8	70.5
DNA TEMPLATE	1	1	1	1	1	0.5	0.5	1
FORWARD PR	5	1.2	1.5	1.6	1.1	1.2	1.5	5
REVERSE PR	5	2.7	2.7	2.7	2.7	2.7	2.7	5
10X RXN BUFFER	18	18	18	18	18	18	18	18
10mM dNTPs	MM							
Varied, microfuze								
Seq. prod	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral oil	100.0					22.9	23.2	
Thermocycler Program								
Step cycle: 30 sec @ 95°C								
30 sec @ 45°C								
3 min @ 72°C								
Repl. cycle: 10 min @ 72°C								
Soak cycle: 4°C								
Master mix (T)								
B, D, F 6 x 10 = 60								
dATP 6 x 2 = 12								
dCTP								
dGTP								
dTTP								
Total 100.0								
Repeated #1, 2, 3 35 cycles								
Thermocycler Program								
Step cycle: 1 min @ 95°C								
30 sec @ 52°C								
3 min @ 72°C								
Repl. cycle: 10 min @ 72°C								
Soak cycle: 4°C								



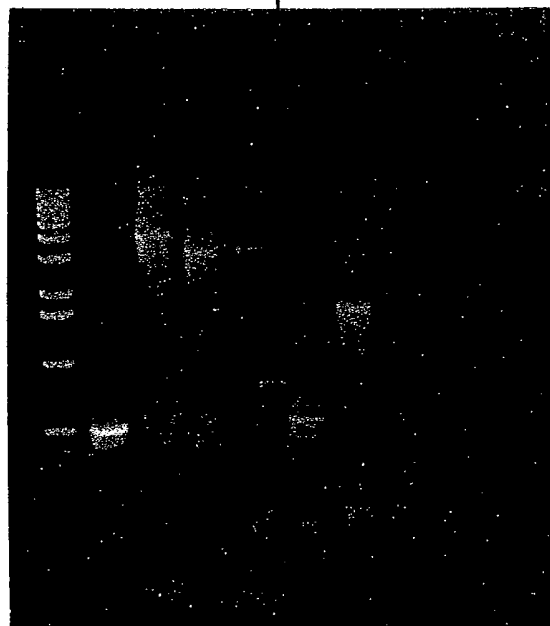
DELETION EXP.

1ST ROUND PCR

8 JAN 93

		1 22.1	2	3	4	VDAM-R2 ⁺²³ 3820-3811
EXPECTED LENGTH (bp)	500	1569	3914	3095	3095	VDB-F1 2292-2119/2301-231
DNA	2 C	ZOWAT 5	ZOWAT 5	ZOWAT 5	ZOWAT 5	Sub labeled VDB-F1
FORWARD PR	P1#1	VDB-F1	VCF1	VE3F1	VE3A2	2301 3820
REVERSE PR	P1#2	VDAM-R2	VAR3	VAR3	VAR3	2119 23
H ₂ O	70.5	76.6	77.1	76.8	76.7	182 3843
DNATEMPERATURE	1	0.5	0.5	0.5	0.5	-2092
FORWARD PR (100 pmol)	5	1.4	1.2	1.5	1.1	1751
REVERSE PR (100 pmol)	5	2.0	2.7	2.7	2.7	-182
MAX PRN DIFFER	12	18	18	18	18	1569
MM						
MM dNTP						
Varied						
Suppl	0.5	0.5	0.5	0.5	0.5	
Minus	100ul					
Step 1: 2 min @ 95°C						
Step 2: 3 min @ 37°C						
Step 3: 3 min @ 72°C						
Step 4: 10 min @ 72°C						
Step 5: 2°C						
Master Mix (T)						
Low buffer						6 x 10 = 60 ul
dATP						6 x 2 = 12
dCTP						" = 12
dGTP						" = 12
dTTP						" = 12

Deletion # 1



24

DUAL A

01

Abs

Factor= 1.000

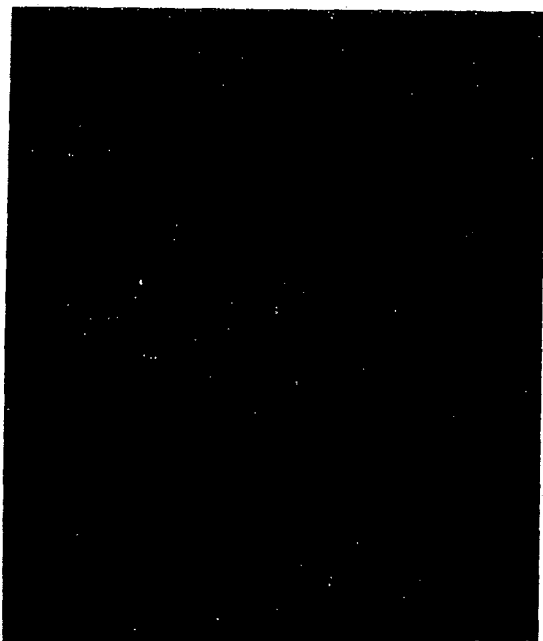
Read average = 01

SW	260.0	280.0	AA/AB	SW	260.0	280.0	AA/AB
1	1.5652	0.7726	2.025	DNA PREP ZOWAT 5 V26 MF1/V26 MR3 - VE3F1/VE2 RZ - bJA			
2	1.9965	1.2893	1.548	" " - VE3F1/VE3R2 - bJA			

#1 78.26 $\mu\text{g/ml}$ = 0.07826 $\mu\text{g}/\mu\text{l}$ = 3.9 $\mu\text{g}/50\text{ml}$

#2 99.825 $\mu\text{g/ml}$ = 0.099825 $\mu\text{g}/\mu\text{l}$ = 4.99 $\mu\text{g}/50\text{ml}$

λ	ABS	SOURCE	11:48	CELL
0.0	2.0101	UV/Vis	01/11/93	sgle



DUAL A

01

Abs

Factor= 1.000

Read average = 01

#	260.0	280.0	AA/AB	SW	260.0	280.0	AA/AB
DNA PREP ZOWAT 5 - V26 MF1/V26 MR3 -							
	0.0003	0.0003	.8782				
	1.8148	1.3649	1.329	PCR PREP - VE3F1/VE2R2 12 JAN - Bam HI / Hind III, EB, CC			
	2.0630	1.5273	1.350	" " VE3F1/VE3R2			

#2 90.74 $\mu\text{g/ml}$ = 0.09074 $\mu\text{g}/\mu\text{l}$ = 4.5 $\mu\text{g}/50\text{ml}$ sample

#3 103.15 $\mu\text{g/ml}$ = 0.10315 $\mu\text{g}/\mu\text{l}$ = 5.1575 $\mu\text{g}/50\text{ml}$ sample

Digestion of VEE

Plasmid 20WATS - V26 MFI/V26 MR3, GB, GC - 22DEC92 amplified
 w/VE3FI/VE2R2 Length = 2,889 bp E3, E2, 6K, E1 (NO ANCHOR)
 + VE3FI/VE3R2 = 1,492 bp E3, E2

VE3FI:

5' > GGT CTA ^{Bam HI} GGA TCC GCC GCC ACC ATG GCT TCA CTA GTG ACC ACC AT
 Kozak 356 972

VE2-R2:

5' > GGT ACT CAA ^{Hind III} AGC TTT CAC TAG GCT GAT CCT CCC ACC AGG CAT G
 STOP STOP 3698

VE3-R2:

5' > GGT ACT CAA ^{Hind III} AGC TTT CAC TAG GCC CGG GCA GTG CCG GCG CAG
 STOP STOP 2281

R.E. digestion:

~~X~~ Hind III - React 2 (50 mM NaCl)
 Bam HI - React 3 (100 mM NaCl)

pBBIII - 20 µg - 1900 92

Add: 86 µl H₂O

10 µl 10X React 2

2 µl ~~Bam HI~~ Hind III

This pBBIII prep apparently is not intact -

PCR PRODUCTS

Add: 36 µl H₂O

10 µl 10X React 2

2 µl Hind III

Inc 1.5 H@ 37°C

Added: ~~2~~ 0.1 µl of 5M NaCl

2 µl Bam HI

3 pBBIII from Mike Parker

1.1 mg/ml = 1.1 µg/µl

Use 11 µg for each of 2 R.E. analysis.

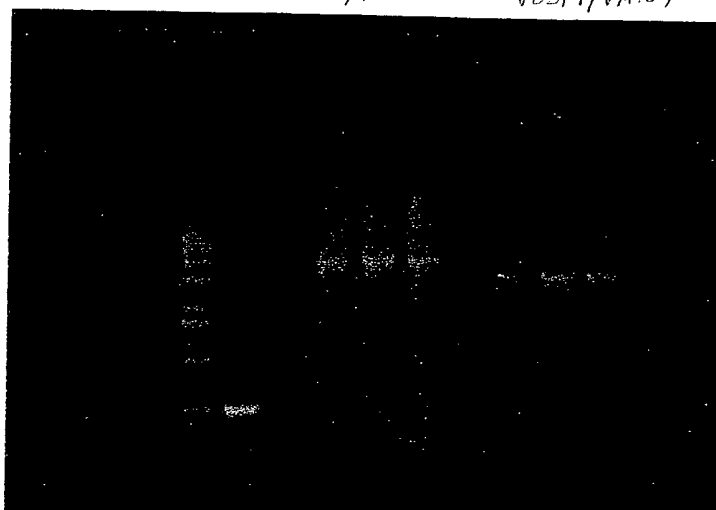
Use 1 run = uncut

1 run = Bam HI + Hind III cut.

Save 5 µl at -20°C to grow up more plasmid.

Amp: 12 Jan 93
 ee Catthip
 notebook 34/60
 p 81
 Gel banded
 Gene-Clear
 p 83
 13 Jan 93
 ee quantitation
 n/p

VE3F1/VAAR3



DUAL	A	01	Abs	Factor= 1.000	Read average = 01	
260.0	280.0	AA/AB	SW	260.0	280.0	AA/AB
0.0162	0.0156	1.038	Blank			
0.0000	0.0000	*****	Blank			
2.8136	1.8168	1.548	pBBIII - HindIII/BamHI, ϕ , pPHCl ₃ , CHCl ₃ , EtOH			

3 140.68 ug/ml = 0.14068 ug/ul = ~7 ug/5 ml. BAP, ϕ /pPHCl₃, EtOH

λ	ABS	SOURCE	08:57	CELL
50.0	2.8158	UV/Vis	01/13/93	sgle

BBIII/H/B/BAP: 0.14068 ug/ul (total 7 ug)
 VE2 R2: 0.09074 ug/ul (total 4.5 ug)
 VE3 R2: 0.10315 ug/ul (total 5.16 ug)

Ligation#	H ₂ O	5X Ligase Buffer	pBBIII/H/B/BAP (0.25 ug)	Insert	VE2	VE3	T4 DNA Ligase	TV (ul)	COLONIES 7500
1	1.8	4	1.8	11 (1:3) (0.75 ug)	—	—	2	20	0
2	—	2	1.8	2.8 (1:1) (0.25 ug)	—	—	1	10	0
3	4.3	2	1.8	0.9 (3:1) (0.08 ug)	—	—	1	10	0
4	19.75	6	0.15 (20 ng)	1.1 (100 ng)	—	—	3	30	1
5	—	2	1.8	—	—	7.2 (1:3) (0.75 ug)	1	12	1
6	2.8	2	1.8	—	—	2.4 (1:1) (0.25 ug)	1	10	2
7	4.4	2	1.8	—	—	0.8 (3:1) (0.08 ug)	1	10	0
8	19.9	6	0.15 (20 ng)	—	—	0.97 (100 ng)	3	30	1
9	5.2	2	1.8	—	—	—	1	10	0

NOTE: PUC - NO COLONIES

Mike Parker ligation receipt:

20 ng vector, 100 ng insert in a 30 ul rxn $\Rightarrow$ 5 ul transformations

NOTE: SIN used 0.5, 1 & 2 ul of 5-10 ng/ μ l rxn with 0.17 ug plasmid.
 1-2 colonies $\Rightarrow$ 2 out of 4 positive

Inc ON @ 16°C

Cathy Lind made competent EOP10' cells and did the transformation of the above ligations

14 JAN 93 / 7:20A Transfer ON culture of TOP10 cells in 2 ml SOB broth to 100 ml of SOB broth in 500 ml Erlenmeyer flask.

	SINGLE λ	01	Abs	Read average = 01
SW	600.0	SW	600.0	SW 600.0
3	0.0077			
14	0.4651			

λ	ABS	SOURCE	09:16	CELL
600.0	0.0084	VIK/UV	01/14/93	1

read at 0830

	SINGLE λ	01	Abs	Read average = 01
SW	600.0	SW	600.0	SW 600.0
1	-0.0000			
2	0.2121			

100 μ l of transformation was added to LB/AMP plts. Inc ON @ 4°C

15 JAN 93 Have 5 colonies:

labeled 4, 5, 6-1, 6-2, 8-1. The 1st # refers to the ligation #. Colonies were transferred to grid plt and 2 ml LB/AMB br.

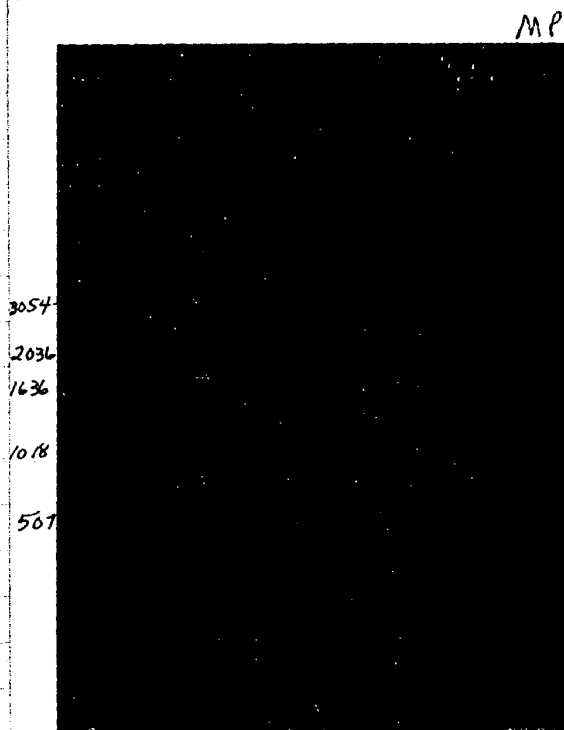
20 μ l of TOP10 transformation was added to LB/AMP plts

Another transformation was done using INV & F1 cells. 100 μ l of the transformation was added to LB/AMP plts.

	DUAL	λ	01	Abs	Factor= 1.000		
					Read average = 01		
SW	260.0	280.0	λA/λB	SW	260.0	280.0	λA/λB
1	0.9152	0.5193	1.762	DNA PREP VCB F/ VCB3 -GB, GC-13 JAN 93			
2	0.7275	0.4777	1.523	" VCB1/VAR3 - " "			
3	1.4194	0.9494	1.495	MP			

#1 $45.76 \mu\text{g/ml} = 0.04576 \mu\text{g}/\mu\text{t} = 2.288 \mu\text{g}/50 \text{ ml sample}$
 #2 $36.375 \mu\text{g/ml} = 0.036375 \mu\text{g}/\mu\text{t} = 1.81875 \mu\text{g}/50 \text{ ml sample}$
 #3 $70.97 \mu\text{g/ml} = 0.07097 \mu\text{g}/\mu\text{t} = 3.5485 \mu\text{g}/50 \text{ ml}$

λ	ABS	SOURCE	11:38	CELL
260.0	1.4024	UV/Vis	01/14/93	sqle



Megaprimer was synthesized by: Deleted 2119 → 2301

DNA template: ZOWAT 5

FORWARD PR: VDB-F1 (2092 - 2118/2302-2316) ⁽²⁶⁾

REVERSE PR: VDA-M-R2 (3830 - 3811 + 23)

3830

23

3853

3274

- 3092

+ 151

= 182

1569

2302 → 3853 = 1551

1551

26

1,577 bp

$$1569 \times 324.5 \times 2 = 1018281 = 1 \times 10^6$$

Need 0.6 pmol of MP

$$\frac{x}{1 \times 10^6} = 0.6 \times 10^6$$

$$x = 0.6 \times 10^6$$

$$= 0.6 \mu g$$

Have 0.071 $\mu g / \mu l$

Add 8.56 μl / ~~1000~~ ¹⁰⁰⁰

ES: Ave MW of deoxynucleotide = 324.5 daltons

of nucleotides $\times 2 \times$ Average MW of deoxynucleotide (324.5) = MW

$$\frac{\mu g}{MW} = \text{pmole DNA}$$

DELETION EXP - STEP 2
14 JAN 93

1 JAN 93

EXPECTED LENGTH	500	~3900
DNA	XC	20WATS
FORWARD PR	Pr #1	MP
REVERSE PR	Pr #2	—
H ₂ O	70.5	12.4
DNA TEMPLATE	1	0.5
FORWARD PR	5	8.6
REVERSE PR	5	—
10X RNABUFFER	18	18
10mM dNTP	MM	
Vortex microfuge		
Taq pol	0.5	0.5
Minisat oil	100	100
Thermocycler Program:		
Step cycle	5 min @ 95°C	1 cycle Total 21
	2 min @ 37°C	
	10 min @ 72°C	
No delay cycle		
Soak cycle 4°C		
MM (I)		
10X RNABuffer	60	
dATP	6x2 = 12	
dCTP	12	
dGTP	12	
dTTP	12	
		108

14 JAN 93

EXPECTED LENGTH bp	500		
DNA	2.0 from STEP 2	STEP 2	20WAT 5
FORWARD PR	Pr #1	VE3-F1	VE3-F1
REVERSE PR	Pr #2	M13	VDAMR2
H ₂ O	—	—	78.8
DNA TEMPLATE	—	—	0.5
FORWARD PR	—	1.5	1.5
REVERSE PR	—	0.72	2.0
10RXN BUFFER	—	—	18
10mM dNTP	—	—	—
Taq pol	—	—	0.5
Thermal oil	—	—	100 _{at 122}
Thermocycler Program			
Step cycle: 2 min @ 95°C			
2 min @ 37°C			
3 min @ 72°C			
Delay: 10 min @ 72°C			
Soak: 4°C			

Year	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099
1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	

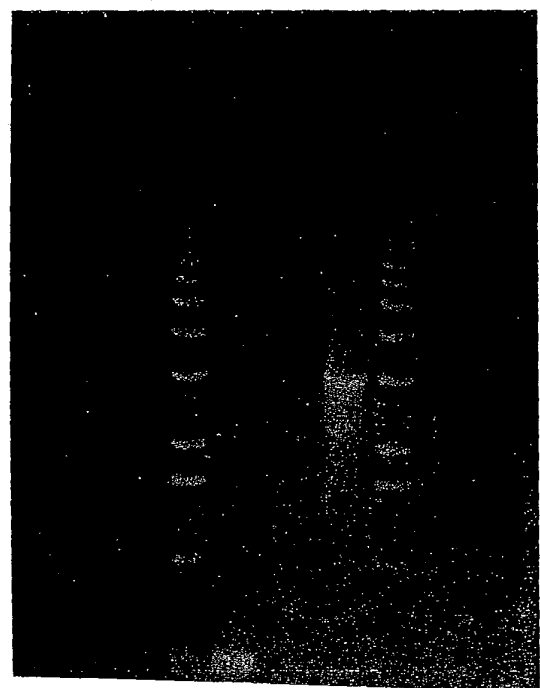
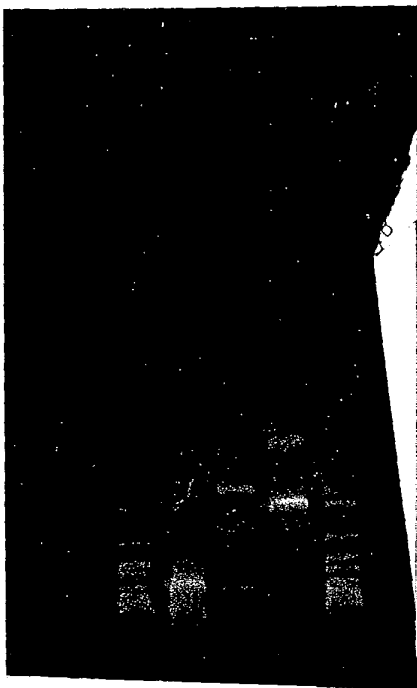
Selection Exp:
 2nd Round PCR
 15 JAN 93

3rd Round PCR
 15 JAN 93

STEP 2

STEP 3

ITEM	AMOUNT	EXPECTED LENGTH	500'	~3000	~3000
NA	ZOWATS	DNA	2C	STEP 2	ZOWATS
VERSE PR	MP	FORWARD PR	P1#1	VE3F1	VE3F1
H ₂ O	71.9	REVERSE PR	PCH2	M13	VDAM R2
TEMP.	0.5	H ₂ O	70	77.8	78.8
VERSE PR	8.6	DNATEMP.	1	1	0.5
(pmol)		FORWARD PR	5	1.5	1.5
RXN	18ul	REVERSE PR	5	0.72	2.0
EFER		10X RXN	18	18	18
MDNTP		BUFFER	MM	2.22	
pol	Vortex, microfuge	10pmMDNTP			
Mineral oil	100	Taq pol	1	1	1
		Mineral oil	100	100	100
Thermocycler Program:		Thermocycler Program			
Step Cycle: File 21		Step Cycle: File 40			
3 min @ 95°C		30 sec @ 95°C			
2 min @ 42°C		30 sec @ 52°C			
10 min @ 72°C		3 min @ 72°C			
Soak: 4°C		Delay: 10 min @ 72°C			
		Soak: 4°C			
Master Mix (T)					





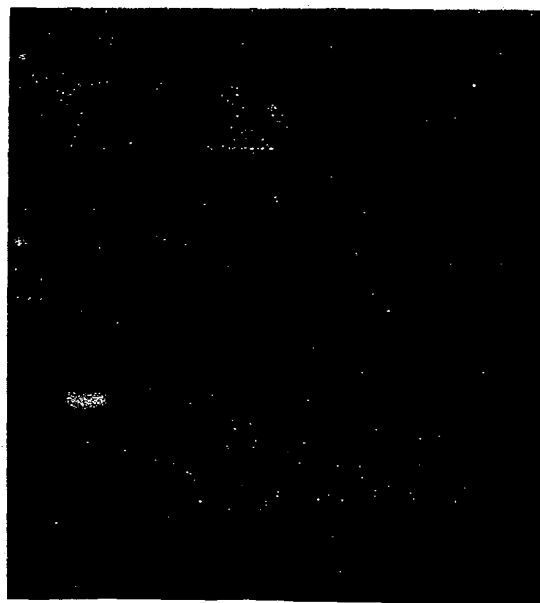
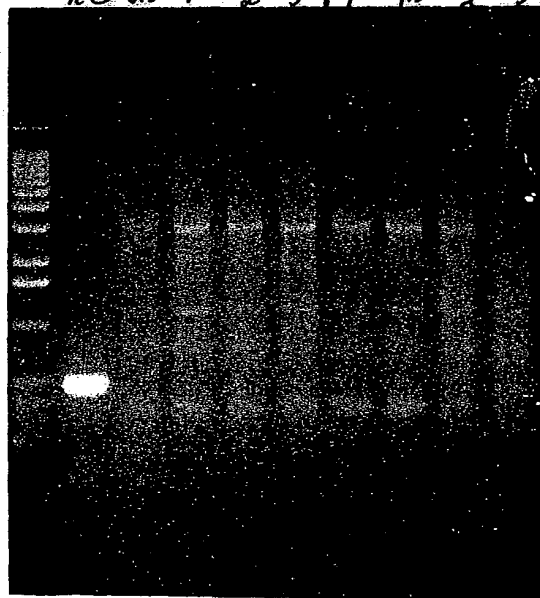
V-DAM-R2 3830-3811/AMXIII-M13
 +21
 VEB-F1 856-832/KOZAL Gm HI
 VDB-F1 2092-2119/2301-2313
 DELETION EXP
 2nd ROUND PCR
 15 JAN 93

8563830
 27 23
 829 3853
 829
 3024

EXPECTED LENGTH		1569							
FO DNA	λC	ZOWATD							
RE FORWARD PR	Pr#1	MP							
REVERSE PR	Pr#2	—							
D H ₂ O	69.5	72.4							
FO DNA TEMPLATE	1	0.5							
RE FORWARD PR	5	8.6	1.0						
10 REVERSE PR	5								
10 B ₁									
10X RXN	7.18	1.8							
10 BUFFER	MM								
10 mM dNTP									
10X pol	Vortex, microfuge								
Thermostad	100	100							
Thermocycler Program:									
2 3 min @ 94°C									
Step cycle: 30 sec @ 94°C									
30 sec @ 50°C									
45 sec @ 72°C									
30 cycles									
Display cycle: 10 min @ 72°C									
Soak 4°C									

16
 16
 2nd
 2nd

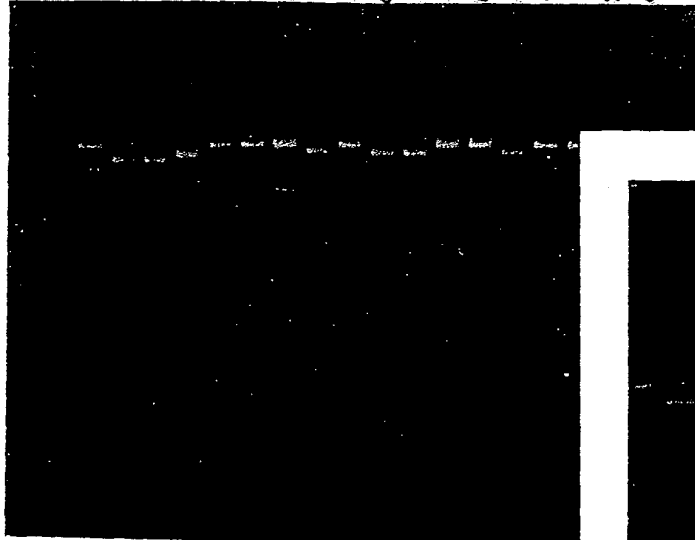
2C 0.5 1 2 3 1.5 2 3 mM



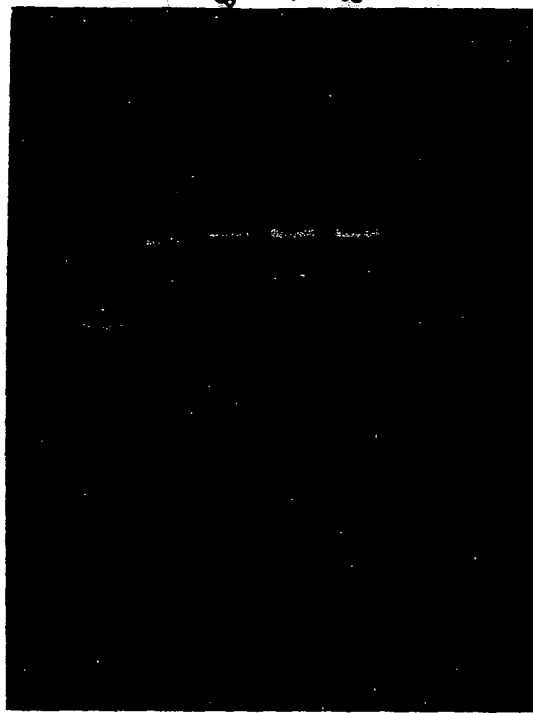
Deletion Exp. MgCl₂ Optimization 20 JAN 93

[MgCl ₂]		1	1.5	2	3			
ONA	2C	STEP 2 PRODUCT (20 WAT 5/MP)						
		15 JAN						
FORWARD PR.	Pr #1	VE3F1	→					
REVERSE PR.	Pr #2	M13	→					
EXPECTED LENGTH (bp)	500	3000	→					
		20 ²²						
H ₂ O (μl)	70	73.8	71.8	69.8	65.8			
DNA TEMPLATE	1	1	1	1	1			
FORWARD PR	5	1.5	1.5	1.5	1.5			
REVERSE PR	5	0.72	0.72	0.72	0.72			
10X RXN BUF	10 (I)							
10 mM dNTP	2 tubes							
25 mM MgCl ₂		4	6	8	12			
	Vortex, centrifuge							
Tag pol	1	1	1	1	1			
Mineral oil	100 μl							
Thermocycler Program:	File #0	Master mix (II)						
Step cycle:		40 cycles						
30 sec @ 95°C								
30 sec @ 55°C								
3 min @ 72°C								
Delay: 10 min @ 72°C								
Soak: 4°C								
						Buffer = 5 x 10 = 50		
						dATP 5 x 2 = 10		
						dCTP 10		
						dGTP 10		
						dTTP 10		
						TV = 90 μl		

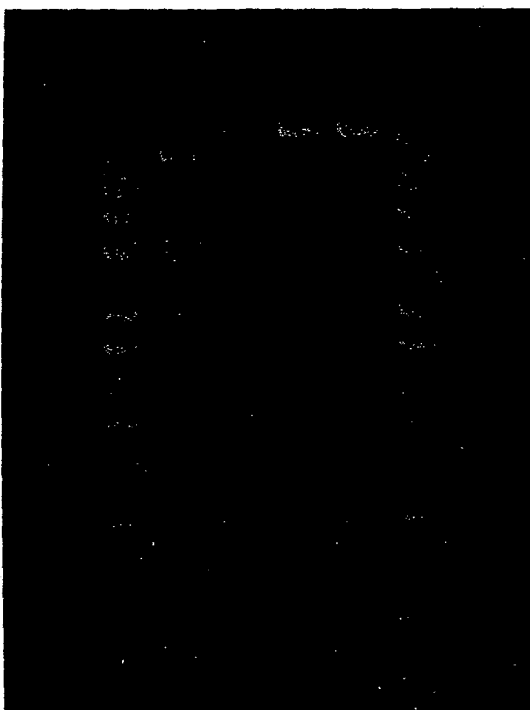
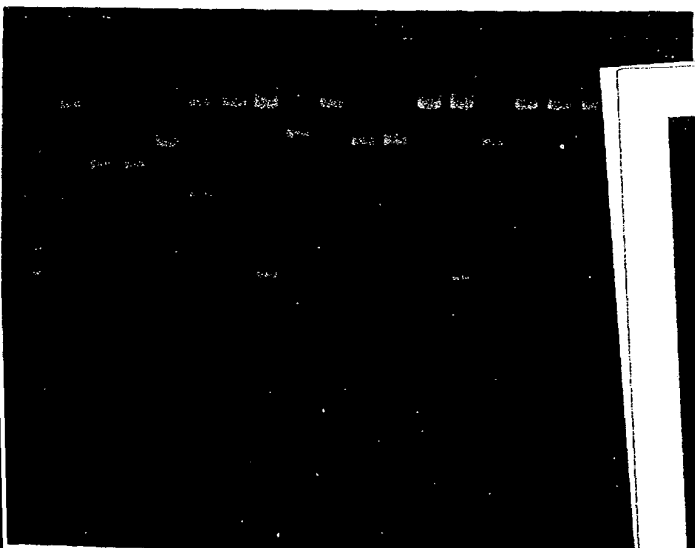
1-1
2-1
3-1
4-1
5-1
5-2
5-3
6-1
6-2
6-3
6-4
6-5
6-6
7-1
7-2
7-3
8-1



8-2
8-3
8-4
8-5



Abboxe gele run OP @ 10V



93 Currently have 5 colonies from LB/AMP plts w/
100 μ l of transformation media.

Fig #

4 +

5 +

6-1 +

6-2 (-)

8 (+)

Have 16 colonies from LB/AMP plts w/ 200 μ l of transformation media

<u>Fig #</u>	<u># colonies</u>	<u>LABEL</u>
1	1	(1-1)
2	2	(2-1), 2-2
3	1	(3-1)
4	0	—
5	2	5-2, 5-3
6	3	6-3, 6-4, 6-5
7	3	7-1, 7-2, 7-3
8	4	8-2, 8-3, 8-4, 8-5
9	1	
pVC	9	
pVC 1:100	0	
Cells only	0	

Transformation w/ INV α cells on 15 Jan 93

<u>Fig #</u>	<u># colonies</u>	<u>LABEL</u>
6	1	6-1

All other plts had 0 colonies

All 22 colonies were MP with Promega system.

RE:

14 μ l H₂O

3 μ l MP DNA

2 μ l 10X React 2 buffer

1 μ l Hind III

Inc 1 H @ 37°C

Add 0.1 μ l 5M NaCl

1 μ l BamHI

Inc 1 H @ 37°C

... .. (Method) on following

DUAL A 01 Abs Factor=1.000

Read average = 01

SN	260.0	280.0	AA/AB	SN	260.0	280.0	AA/AB
5 ut in 500 ut H ₂ O dil = 1:101 500 ut total sample							

1	0.0001	0.0001	1.000
2	1.0435	0.5746	1.816 VEE SP-6 (1-1)
3	1.0574	0.6273	1.685 VEE SP-6 (4-1)
4	0.1247	0.0660	1.889 VEE SP-2 (5-2)
5	1.6223	0.8471	1.915 VEE SP-2 (6-5)

#2 5,269.615 ug/ml = 5.3 ug/ut = 2.65 mg/500 ut

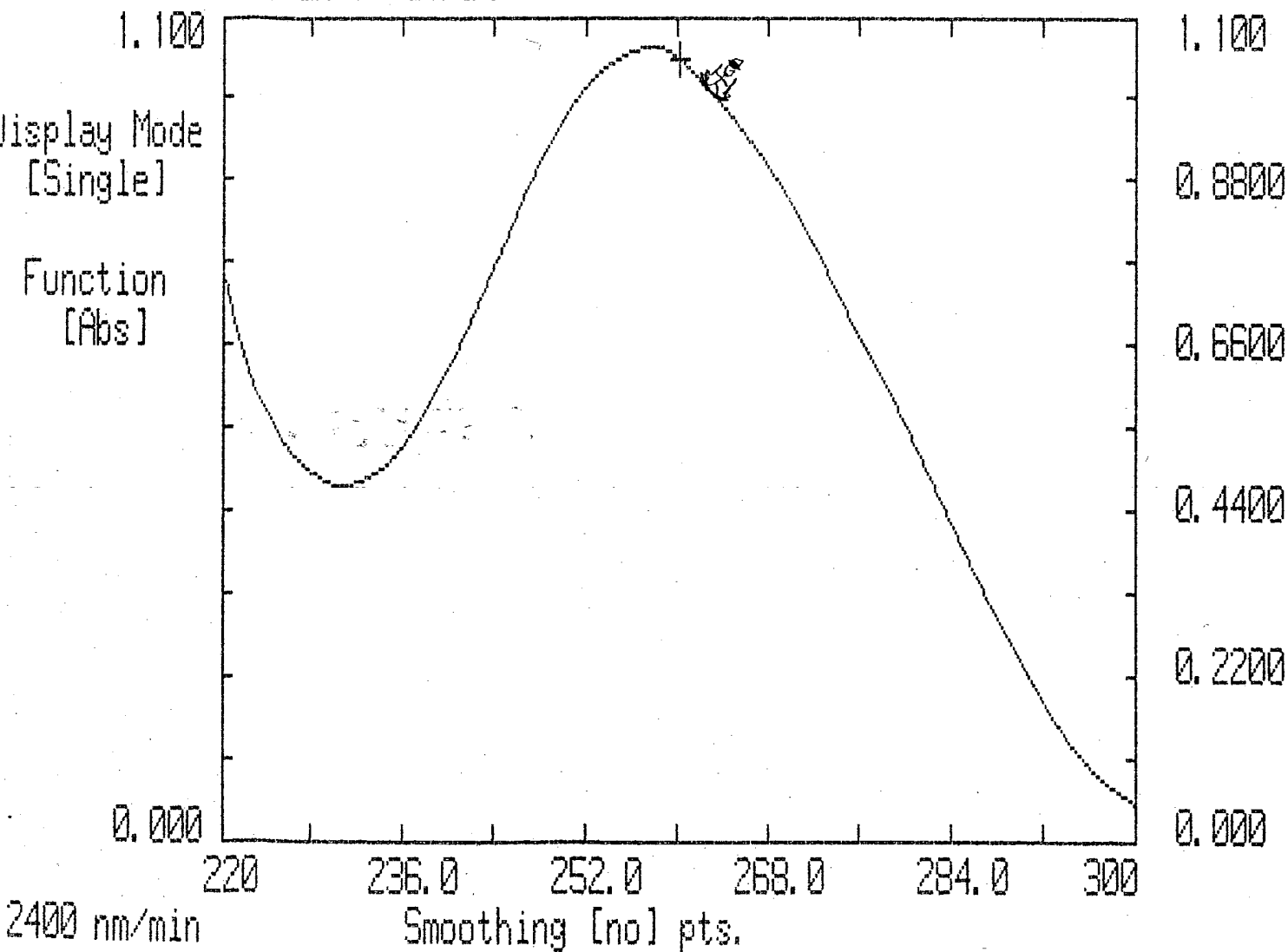
#3 5,339.81 ug/ml = 5.3 ug/ut = 2.65 mg/500 ut

#4 629.735 ug/ml = 0.63 ug/ut = 0.315 mg/500 ut

#5 8192.615 ug/ml = 8.19 ug/ut = 4.1 mg/500 ut

λ	ABS	SOURCE	12:01	CELL
260.0	0.0000	UV/Vis	01/22/93	1

Scan # 01.02 VEE SP-6 (1-1)



4143 R.E.:

	DNA (mapprep)-10ug	H ₂ O (ul)	10X React 2 (ul)	Hind III (ul)
1-1 =	2 ul	15	2	1
4-1	2	15	2	1
5-2	15	2	2	1
6-5	1.25	15.75	2	1

Inc 1H @ 37°C

Add: 0.1 ul 5M NaCl

1 ul Bam HI

Inc 1H @ 37°C

Ice 10 min

Entire sample on gel.

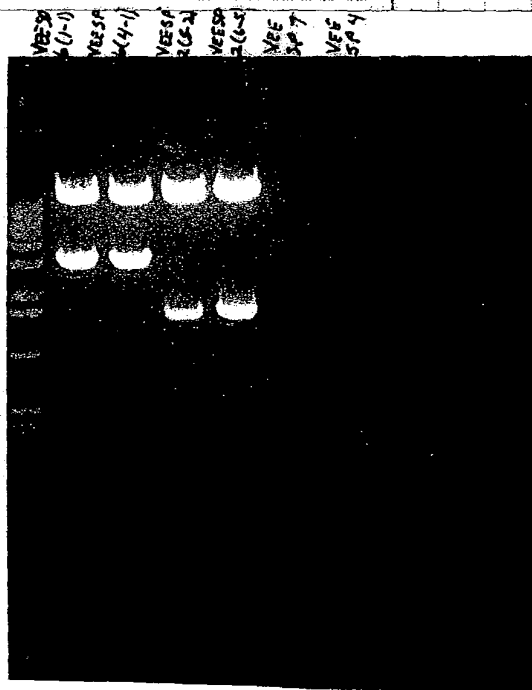
DUAL A 01 Abs Factor = 1.000
 GB, GC 5X AMP 20WAT/V26MFI- Read average = 01
 SW 260.0 280.0 AA/AB SW 260.0 280.0 AA/AB

1 0.5882 0.3365 1.747 VCF1/ VAR3
 2 0.9635 0.5717 1.685 VEF3F/ VAR3 } 135AN93

#1 29.41 ug/ml = 0.02941 ug/ul 1.4705 ug/50 ml sample.
 #2 48.175 ug/ml = 0.048175 ug/ul 2.41 ug/50 ml sample

VEE SP 76
 VEE SP 46
 3.0

A ABS SOURCE 14:30 CELL
 260.0 0.9637 UV/Vis 01/22/93 sgls



Maxipreps:

VEE SP-6 (1-1) 5.3 $\mu\text{g}/\mu\text{t}$
 VEE SP-2 (6-5) 8.19 $\mu\text{g}/\mu\text{t}$
 S/N# 1 4.6 $\mu\text{g}/\mu\text{t}$
 pBB III DNA 1.1 $\text{mg}/\text{ml} = 1.1 \mu\text{g}/\mu\text{t}$
 WT linear DNA only —

0.6 $\mu\text{t} = 3 \mu\text{g}$
 0.4 $\mu\text{t} = 3 \mu\text{g}$
 0.7 $\mu\text{t} = 3 \mu\text{g}$
 2.7 $\mu\text{g} = 3 \mu\text{g}$

Have 1. 3×10^6 cells / ml

Need 2 ml of TNM-FH complete media for each transfection. No duplicates. Need 10 plts.

$10 \times 2 = 20 \text{ ml}$. - Make up enough for 22 ml.

Need 10 (2×10^6) cells = 22×10^6 cells total

Add 17 ml of cells + 5 ml of media.

The cells are in SS-900 media. Will let cells attach in this media also.

Glycerol Stocks:

Bacterial cultures grown in liquid media:

To 0.85 mL of bacterial cultures, add 0.15 mL sterile glycerol (sterilized by autoclaving for 20 min. at 15 lb/sq. in. on liquid cycle).

Vortex the culture to ensure that the glycerol is evenly dispersed. Transfer the culture to a labeled storage tube equipped with a screw cap and air tight gasket. Freeze the culture in ethanol-dry ice and then transfer the tube to -70C for long-term storage.

Appendix A.5 - Maniatis, 2nd ed., Vol. 3

Inoculate 2 - LB/TET plts w/ TOP 10F'

Sud 2×10^6 SS9 cells in ~~complete~~ ^{SS900} FNM-FH in a 60mm plate. Allow the cells to attach for at least 30 min.

2. For each 60mm plate to be transfected set up the following transfection mix. Pipette 1 ml of Grace's media (Cat # B823-01) containing no supplements into a 1.5 ml sterile eppendorf. Add 1 μ g of linear Ac MNPV DNA (Cat # B825-03) and 3 μ g of plasmid DNA (the transfer vector containing the gene of interest). Thoroughly mix the cationic liposome solution then add 50 μ l of the DNA/Gace's media solution. Vortex vigorously. See previous page (p 42) for samples to be transfected.

3. Incubate at RT for 15 min.

4. During this incubation period remove the media from the plate and replace with 2 ml of Grace's media w/o supplements added. Allow the cells to sit until the incubation in Step 3 is within 2 min. of completion. This should be approximately 10 min.

5. Remove all of the Grace's media from the cells and add the 1 ml transfection dropwise to the 60 mm plate.

6. Incubate plates on a slowly rocking platform at RT for 4 H. (Setting = 2.5 on Belleo sid/sid rocking platform.)

7. Following this incubation period add an additional 1 ml of ~~complete~~ ^{SS900} FNM-FH media to each 60 mm plate and incubate at 27°C in a humidified environment for 48 H.

8. 48 H later harvest the media from the transfected plates and store at +4° until required. This media is the virus stock which will be used in the

50 mg/ml X-gal ^{in blue} in dimethylformamide
 Make up 2 ml = 200 mg

Streptomycin:

$$50,000 \times = 100 \cdot 50$$

$$\times = 0.1 = 100 \mu\text{t in } 100 \text{ ml media}$$

fungicide

$$250 \times = 2.5 \cdot 100$$

$$\times = 1 = 1 \text{ ml in } 100 \text{ ml media}$$

ligations of VEE SP-4 and VEE SP-7
R products.

E3F1/VAR 3 - 13 JAN 93 = VEE SP-4 (3095bp) = 0.048175 μ g/ μ l

E3F1/VAR 3 - " = VEE SP-7 (3,414bp) = 0.02941 μ g/ μ l

BBIII/H/B/GAP = 0.14068 μ g/ μ l

MAN BE	H ₂ O	5X LIGASE BUFFER	pBBIII/ H/B/GAP 0.25 μ g	INSERTS	VEE SP-4	VEE SP-7	T4 DNA LIGASE	TV (μ l)	LB/ AMP 28 JAN
1	2.2	6	1.8	15.6 (1:3 = 0.75 μ g)	17 (1:2 = 0.5 μ g)	3	30	1	
2	3.7	4	1.8	5.2 (1:1 = 0.25 μ g)	8.5 (1:1 = 0.25 μ g)	2	20	0	
3	2.5	2	1.8	1.7 (3:1 = 0.08 μ g)	2.7 (3:1 = 0.08 μ g)	1	10	1	
4	17.45	6	0.15 (20ng)	2.1 (100ng)	3.4 (100ng)	3	30	1	
5	3.6	6	1.8	15.6 (1:3 = 0.75 μ g)	—	3	30	0	
6	—	2	1.8	5.2 (1:1 = 0.25 μ g)	—	1	10	0	
7	3.5	2	1.8	1.7 (3:1 = 0.08 μ g)	—	1	10	2	
8	18.75	6	0.15 (20ng)	2.1 (100ng)	—	3	30	1	
9	5.2	2	1.8	—	—	1	10	0	
Cells only									

Cells only

Plaque Purification of Recombinant virus

Have 5 transformation reactions

1 - VEE SP-2

2 - VEE SP-6

3 - SJV #1

4 - pBBIII DNA

5 - WT linear DNA only

Will do 4 plts for each

2 - dilutions

1 - X gal

1 - Bluegal

20 plts
total

Will set up plaque assays in duplicate - add
X-gal to 1 set of plates and Bluegal to 1 set
Add 3.75 g of baculovirus agarose / 150 ml of
Millipore H₂O. (This is a 2.5% (w/v) solution in
distilled H₂O). Autoclave to dissolve and to sterilize
the solution. Incubate at 50°C until needed.

5 ml of this solution used per 100 mm plate.

Add 50 μ g/ml gentamicin and 2.5 μ g/ml
amphotericin B to SS 900 media. Make up 100 ml
of this media =

Gentamicin STOCK = 50 mg/ml

Amphotericin B (Fungizone) = 250 μ g/ml

Need concentrations of

Gentamicin : 50 μ g/ml

Fungisone : 2.5 μ g/ml

stock

50 mg

$$50,000 \cdot x = 150. \cancel{40} \cancel{60}$$

$$= \cancel{10500} 9000$$

$$x = \frac{9000}{50,000} = 0.18 = 0.18 \text{ ml}$$

$$1 \text{ ml} \times 20 \text{ mg} = 20 \text{ mg}$$

$$20,000 \cdot x = 150.60$$

$$20,000 x = 9000$$

$$x = 0.45 = 0.45 \text{ ml}$$

450

10^{-2} - 500 μ l of 10^{-1} + 4050 μ l SF900 media.

Vortex virus inoculum completely before adding to cells.

5. Seed SF9 cells at a density of 8×10^6 cells / 100 mm plate in complete media.

Have Need 20 pfts.

100 $\times 10^6$ cells total

Have 1.92×10^6 cells / ml.

Add 52 ml of cells in 100 ml media (48 media)

Have 5 - LAB tek slides to set up

Need 0.5×10^6 cells / 8 well LABTEK = ~~2.5~~ 3.0×10^6 cells

400 μ l / 8 well ~~slide~~ = ~~2000~~ 3.2 ml / 8 well slide.

Make up 3×10^6 cells in 19.2 ml media.

Have 1.92×10^6 cells / ml

Add ~~1.56~~ 1.6 ml cells + 17.6 ml media

Lock plates at RT on a side/side platform in order to distribute the cells evenly. Use a setting of 5 on Bellco rocker. Incubate for at least 30 min.

Examine the cells to confirm that cells have attached.

Remove plates from the rocker and sit at R.T. for at least 30 min before using.

6. For each 100 mm plate, place 5 ml of SF900 complete media + 150 μ g/ml of Blugalar X gal -

~~20~~ ml media + 210 μ l of chromogenic substrate into a sterile 15 ml Falcon tube. Set aside until needed.

7. Remove all but 2 ml of media from the cells once they have firmly attached. (Remove 3 ml)

8. Add ~~1~~ 1μ l of each dilution dropwise to the appropriately labelled plate. Following addition of the viral dilutions incubate the plates at room temp on a slowly rocking platform for 1 H. (2.5 setting on Bellco)

9. Just prior to the end of the 1 H incubation retrieve the autoclaved agarose and media that had been incubated at 50°C . Combine 1 bottle of agarose to 1 bottle of media, mix & place at 46°C .

At the completion of the incubation, period, begin

this immediately by inverting. Then pour the mixture onto one of the plates from which media was removed.

Incubate plates for 5-6 da in a 27°C incubator w/ high humidity.

[FA]

93 Have 1 ~~slide~~ LAB-TEK slide w/ transformants infected Sf9 cells from 26 JAN 93.

Add 75 μl of primary antibody preparation to respective wells:

VEE = 1:100 (This is a glycerol stock which is 50% glycerol)

SIN = 1:200

Make up dilutions in 20% FBS in PBS.

CV filter solutions before use.

VEE = 10 μl of STOCK + 900 μl 20% FBS/PBS

SIN = 5 μl of STOCK + 950 μl 20% FBS/PBS

Inc 1 H @ RT in humidifying tray.

Wash 3X with PBS after aspirating antibody solution from slides.

Add 1:75 dil of FITC conjugate (K+P Cat#) goat anti-mouse to each slide.

Inc 1 H @ RT in humidifying tray.

Wash 3X w/ PBS after aspiration of 2^o antibody.

Air dry

Coverslips using anti fade mounting media.

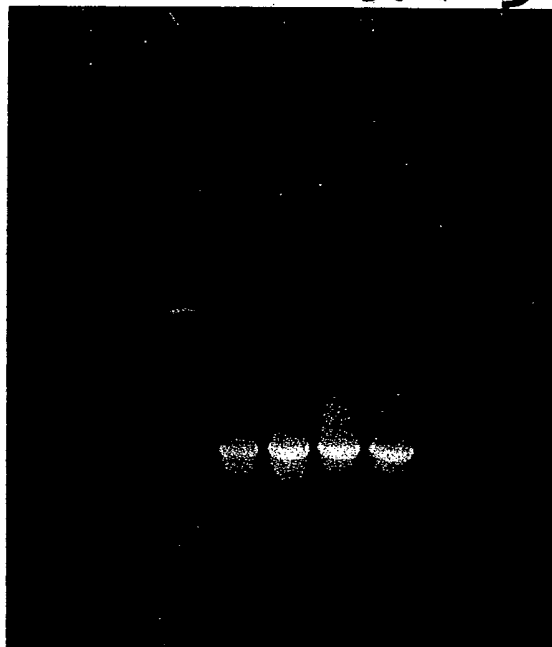
[MgCl₂]23.3
28 JAN 93gation - (1st ROUND
done on 8 JAN 93
p 21 this notebook)

VEE SP-3

→	LC	20WAT PCR PRODUCT	5' / V26 MFI / GB, GC	V26 M R2 TJAN 93	V6K-F1 VAR 3	2302-3911 -3911	2320-24 +14
PR	Pr#1	V6K-F1	→	→	3911		
PR	Pr#2	VAR 3	→	→	2302		
ED	500	1647	→	→	1609		
H(bp)					38		
	70.5	72.7	70.7	68.7	64.7		
PLATE	1	1	1	1	1		
PR	5	1.1	1.1	1.1	1.1		
PR	5	2.7	2.7	2.7	2.7		
BUFF	10	18	18	18	18		
INTP	2nd last dATP						
13	4	6	8	12			
al	0.5	0.5	0.5	0.5	0.5		
al	100 ul						
cycle - program							
cycle: 30 sec @ 95°C							
30 sec @ 52°C							
3 min @ 72°C							
lay cycle: 10 min @ 72°C							
lay cycle: 1°C							
	29.3	31.3	35.3				

Master Mix: 10X Rxn buffer 6 x 10 = 60
 dATP 6 x 2 = 12
 dCTP " 12
 dGTP " 12
 dTTP " 12

VEE SP 3



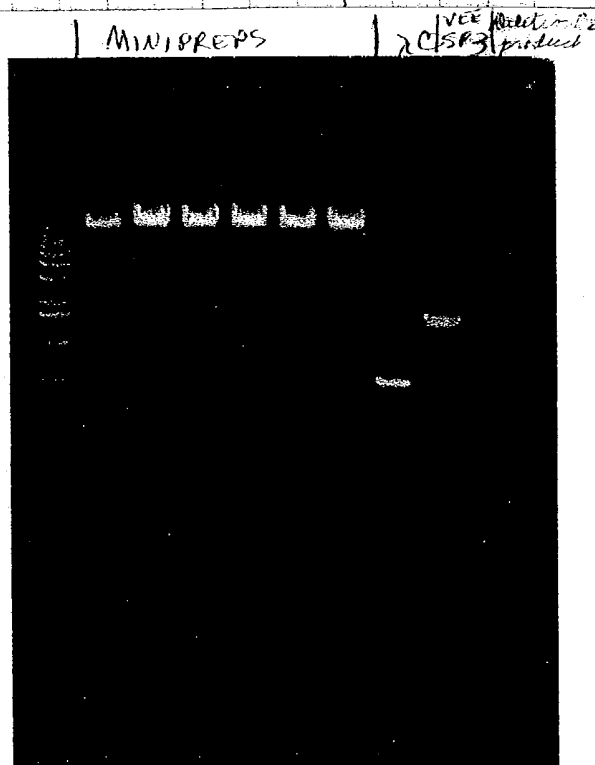
gel
 lanes
 W
 markers

Minipreps were done by Cathy Lind on the 6 colonies from the transfection of 26 JAN 93 (see p 45 this notebook)

There were no inserts.
After review of procedure - it was found that the inserts had not been digested - experiment discarded.



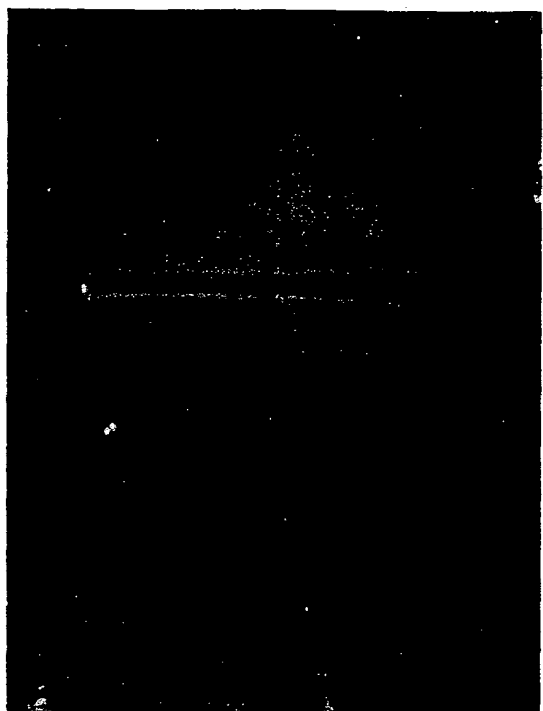
N.E. SP 3.1
5X Amplification
29 JAN 93



DNA	λC	ZOWAT 5/V26MFI/V26MR2	PCR PRODUCT - G.B, GC - 75 JAN 95
1/		[1X]	[5X]
FORWARD PR	Pr#1	V6 E-F1	
REVERSE PR	Pr#2	VAR3	
EXPECTED LENGTH (bp)	500	1647	
H ₂ O (ul)	10.5	68.7	343.5
DNA TEMPLATE	1	1	5
FORWARD PR	5	1.1	5.5
REVERSE PR	5	2.7	13.5
10X RNase BSA	10	18	90
10mM dNTP	2 ul ea	2 ul ea	
25mM MgCl ₂	—	8	40
Topo pol	0.5	0.5	2.5
Minerabul	100		500
Thermocycler Program			
Stop time:			

Master Mix (II)
10X Run buffer = 6 x 10 = 60

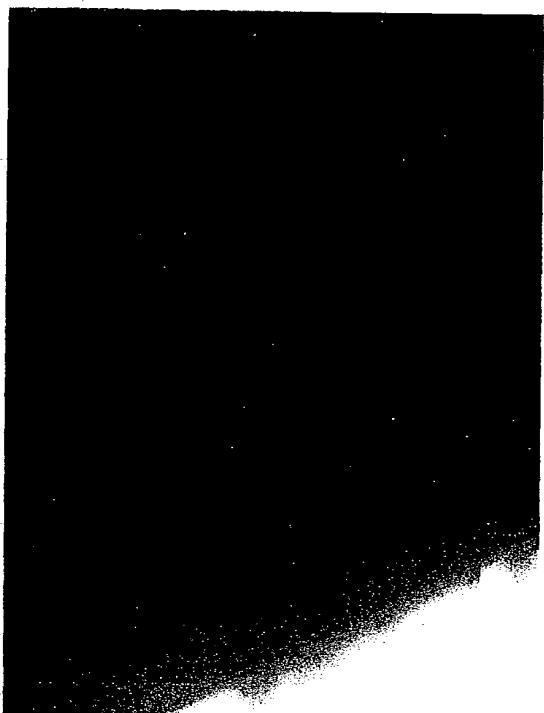
Mel-binding, Gene Clean of VEE SP3, VEE SP4, VEE SP7



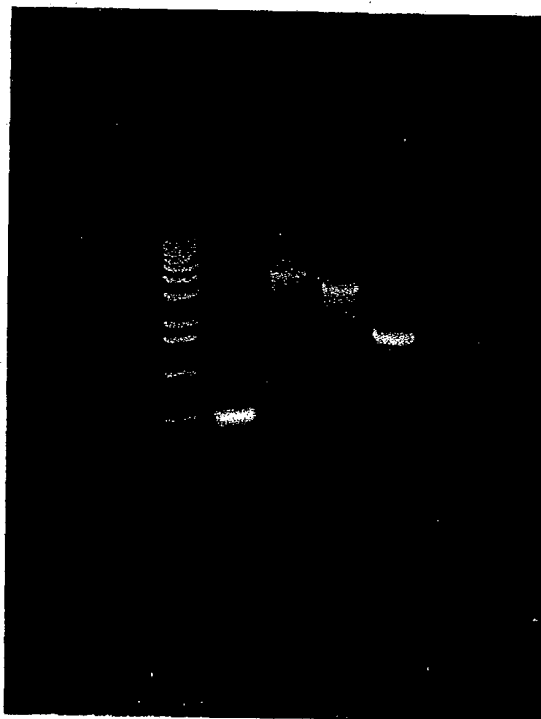
30 JAN 93 VEE SP3
1647 bp



VEE SP4 30 JAN 93 VEE SP4
3095 bp



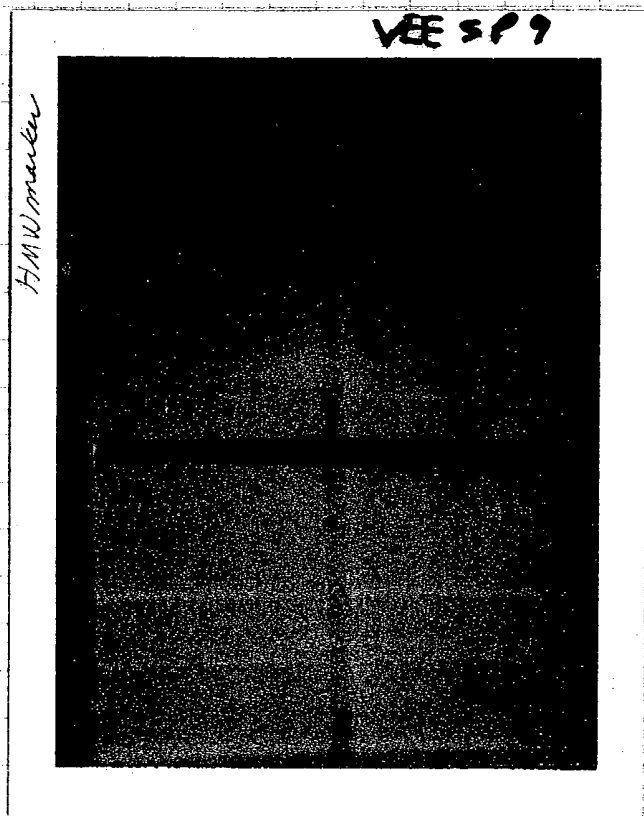
VEE SP7 30 JAN 93 VEE SP7
3,914 bp



5X amplification products

Deletion Experiment:

Have pooled the PCR products from $[MgCl_2]$ optimization experiment and primer/template optimization experiments for gel-banding and Gene-Cleaning for use as template for further amplification of VEE SP9.



2 μ l of the Gene-Cleaned product was run on 1% agarose gel on 29 Jan 93. See p 53 this notebook.

DUAL λ OI ADS Factor = 1.000
Read average = 01

SW	260.0	280.0	$\lambda A/\lambda B$	SW	260.0	280.0	$\lambda A/\lambda B$
GB, CC - 50 μ l samples							
1	2.4565	1.4066	1.746	VEE SP-3	30 JAN 93		
2	0.2633	0.1414	1.862	VEE SP-7	13 JAN 93		
3	1.2078	0.6694	1.804	VEE SP-4	30 JAN 93		
4	0.7401	0.5359	1.381	VEE SP-4	13 JAN 93		
5	0.8913	0.4448	2.003	VEE SP-7	30 JAN 93		

#1 $122.825 \mu\text{g}/\text{ml} = 0.1228 \mu\text{g}/\mu\text{l} = 6.1 \mu\text{g}/50 \mu\text{l}$
 #2 $13.165 \mu\text{g}/\text{ml} = 0.013165 \mu\text{g}/\mu\text{l} = 0.66 \mu\text{g}/50 \mu\text{l}$
 #3 $60.39 \mu\text{g}/\text{ml} = 0.06039 \mu\text{g}/\mu\text{l} = 3.02 \mu\text{g}/50 \mu\text{l}$
 #4 $37.005 \mu\text{g}/\text{ml} = 0.037 \mu\text{g}/\mu\text{l} = 1.85 \mu\text{g}/50 \mu\text{l}$
 #5 $44.565 \mu\text{g}/\text{ml} = 0.045 \mu\text{g}/\mu\text{l} = 2.23 \mu\text{g}/50 \mu\text{l}$

Restriction Digest of VEE SP-3, VEE SP-4, VEE SP-7, pBBIII (Invitrogen)

VEE SP-3 = 6.1 μ g / 50 μ lVEE SP-4 13 JAN 93 = 1.85 μ g / 50 μ l -VEE SP-4 30 JAN 93 = 3.02 μ g / 50 μ l - ~~used~~VEE SP-7 13 JAN 93 = 0.66 μ g / 50 μ lVEE SP-7 30 JAN 93 = 2.23 μ g / 50 μ l.Restriction Endonuclease: 100 μ l reactions

To each of 4 tubes add:

10 μ l Restr 2 buffer (10X)2 μ l Hind III7.2 μ l~~VEE SP~~VEE SP-3: 50 μ l PCR product (6.1 μ g)30 μ l H₂OVEE SP-4: 50 μ l 30 JAN 93 (3.02 μ g) > 4.13 μ g30 μ l 13 JAN 93 (1.11 μ g)VEE SP-7: 50 μ l 30 JAN 93 (2.23 μ g) > 2.62 μ g30 μ l 13 JAN 93 (10.39 μ g)pBBIII: 80 μ l H₂O

Inc 1.5 h @ 37°C

Add: 0.1 μ l 5M NaCl2 μ l Bam HI~~Invitrogen~~ 1-800-955-6288Baculovirus Transfer
Vector pBlueBacIII
10 μ g
Lyophilized
Lot #21116 Store @ -80°C

pBBIII

Heat 65°C for 20 min. Quench on ice for 10 min.

Extract 1X w/ ϕ /CHCl₃/isoamyl alcohol (25:24:1). Vortex.

1 min. Microfuge 12000 x g for 2 min. Back extract w/

100 mM Tris-HCl, pH 8.0. CHCl₃ extract 2X.Precipitate w/ 1/10 volume 3M NaOAc, pH 5.2 and 2 volumes
EtOH.

Freeze on dry ice for 15 min. Microfuge 15 min @ 4°C.

Wash pellet w/ 100 μ l 80% EtOH. Freeze on dry ice for

5 min. Microfuge for 5 min @ 4°C. Air dry on edge of fume

Resuspend in 80 μ l of H₂O. Add 10 μ l 500 mM Tris-HCl,pH 8.0, and 2 μ l BAP (150 U/ μ l). Inc. 1 h @ 65°C.Stop reaction by adding 1 μ l of 0.5 M EDTA. Phenol
extract 1X, CHCl₃ extract 1X, EtOH precipitate as above.

GIBCO BRL

LIFE TECHNOLOGIES, INC.
GAITHERSBURG, MARYLAND 20898

5224SB

Lot No. CH1101

T4 DNA Ligase

100 units; 1 U/μl

Store at -20°C (not frost-free)

Description:

T4 DNA Ligase can be used to join DNA fragments with staggered or blunt ends and to repair nicks in double-stranded DNA having 3'hydroxyl and 5'phosphate ends. The enzyme is isolated for E. coli lambda lysogen NM989.

Unit Definition:

One (Weiss) unit catalyzes the exchange of 1 nmol ³²Pi into [γ/β-³²P]ATP in 20 minutes at 37°C.

Components:

5224SB	T4 DNA Ligase	Lot No. <u>CH1101</u>
Y90001	5X DNA Ligase Buffer	Lot No. <u>CCA106</u>

Storage Buffer:

10 mM Tris-HCl (pH 7.5)
50 mM KCl
1 mM DTT
50% (v/v) glycerol

5X DNA Ligase Reaction Buffer:

250 mM Tris-HCl (pH7.6)
50 mM MgCl₂
5 mM ATP
5 mM DTT
25% (w/v) polyethylene glycol-8000.
Store buffer at -20°. Due to the presence of dilute ATP, the shelf life of the buffer is 6 months.

Note:

FOR OPTIMAL TRANSFORMATION, DILUTE THE LIGATION REACTION ≥ 5-FOLD PRIOR TO ADDING TO COMPETENT CELLS².

Quality Control Data: See Back Page

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE IN HUMANS OR ANIMALS.

EW 05/05/92

Virology
BLV

1-800-828-6682

3:1 molar
ratio

0.1 U -

20 min reaction

VEESP-3
VEESP-4
VEESP-7

Ice 10 min after digestions.
Gel band on 1% LE agarose
Gene-Clean

Quantitate $A_{260/280}$

DUAL	λ		01	Abs	Factor = 1.000	Read average = 01	
SH	260.0	280.0	λA/λB	SH	260.0	280.0	λA/λB
	B/A/C/B/C 1 FEB 93 - 50ul sample						
1	0.0004	0.0000	15.67	BLANK			
2	0.8286	0.4691	1.766	VEESP3			
3	0.4837	0.3047	1.587	VEESP4			
4	0.3599	0.2181	1.649	VEESP7			

#2 $41.43 \mu\text{g/ml} = 0.04143 \mu\text{g/ul} = 2.07 \mu\text{g/50ul}$
 #3 $24.185 \mu\text{g/ml} = 0.0242 \mu\text{g/ul} = 1.21 \mu\text{g/50ul}$
 #4 $17.995 \mu\text{g/ml} = 0.018 \mu\text{g/ul} = 0.9 \mu\text{g/50ul}$

λ	ABS	SOURCE	06:43	CELL
260.0	0.3667	UV/Vis	02/02/93	sgle

Negations		pBBIII / SP 3, 4, 7		Inserts			TV (ul)
Ligation #	H ₂ O	5x ligase buffer	pBBIII (0.25ug)	VEE-SP3	VEE-SP4	VEE-SP7	
1	1.2	6	1.8	18 (0.75ug)	—	—	30
2	18.45	6	0.15 (20ng)	2.4 (100ng)	—	—	30
3	—	6	1.8	—	20.7 (0.5ug)	—	30
4	16.75	6	0.15	—	4.1 (100ng)	—	30
5	—	6	1.8	—	—	22.2 (0.4ug)	30
6	15.25	6	0.15	—	—	5.6 (100ng)	30
7	19.2	6	1.8	—	—	—	30

inc. ~~at 42°C~~ RT for H. Then 4°C 8N. Then 16' till 1:00.
 No colonies on LB/AMP plts with transfections from 2 FEB 93.

Made more competent ^{TOP10} cells. Used ligations from 2 Feb 93 in addition to ligations from Sig 1, 4, 7 and 26 Jan 93 as controls.

SP-31

Colony LB/AMP plate from 2 FEB 93 that was used for miniprep was transferred to LB/AMP grid plate and 2 ml of LB/AMP broth. (There was no grid plate set up when original minipreps were set up so decided to make sure ^{colony} ~~prep~~ still had insert and was viable. Transferred loop of ~~med~~ LB/AMP broth from ON culture for colony isolation on LB/AMP plate. Inc ON @ 37°C. Transferred 1 colony to 3 - 2 ml LB/AMP broths and to LB/AMP grid plate. Inc ON @ 37°C.

~~LB/AMP~~ tubes

1 - used for glycerol stock

2 - used for miniprep

3 - used for inoculum for maxiprep.

Quantitation of Maxiprep $\lambda_{260/280}$ - P. 85 of this notebook

Maxiprep: SP-3

DUAL	A	01	Abs	Factor = 1.000			
Read average = 01							
S#	260.0	280.0	$\lambda A / \lambda B$	S#	260.0	280.0	$\lambda A / \lambda B$
Sub in 500 μ l H ₂ O = 1:101, 500 μ l total volume dil							
1	-0.0001	0.0000	-1111				
2	0.1160	0.0729	1.590				

- Maxiprep SP-3

- maxiprep SP-3

$$585.8 \mu\text{g/ml} = 0.59 \mu\text{g}/\mu\text{l} = 292.9 \mu\text{g total sample}$$

A	Abs	SOURCE	WAVE	CELL
260.0	0.0000	UV/11		

2 Feb 93

FA

Have the following samples:

V-1-1
V-1-2
C-29-1
C-29-2
SIN#1
SIN#2

2 D Harvest

VEE SP-2
VEE SP-6
SIN#1

2 D Harvest
50 "
TD
Inoc from FA (to DA harvest)

Abs for FA: VEE

5A3-1-2
9E12-1-2
11E10-1-2

McAbs from George

SL-K-42 (AF: 1:100)

3B4C-4

1A3A-9

1A3B-7

SIN 4A10-4 (EZ

VEE

HMAF-1:200

CCHF

5F4-1-1

16C2-1-1

16E5-1-1

29D3-1-1

52C0C-CC1AC06

A A07

S G2-1-1

18 F2-1-1

CCHF HMAF 1:200

from Bill
Pitt
AF=1:100
AFS

AF
1:100

0.5 x 10⁶ cells/slide SF9 cells in 3.2 ml media / slide

Made 1:10 dilutions of all of the above samples.

Added 50 μ l to each of the infect.

Results of these slides are in Cathy Lind notebook p99

24-well plates

Seed 6 x 10⁵ cells/well

Set up 12 wells: 432 x 10⁵ cells total = 43.2 x 10⁶ cells

Have 1 x 10⁶ cells/ml STOCK (Passed 29 FEB 93)

43.2 ml of cells + 100.8 ml media

Media: SF900

+ GENT

+ FUNG

+ 1% L-GLUTAMINE

Added 200 μ l of the 1:10 dilutions used for the immediately above this. 3 plates were set in identical fashion.

Media was removed from all wells of the plate set up on 2 Feb 93. 200 μ l of w/o cysteine + 0.5% FBS was added. All media was again removed from and replaced w/ Grace's media w/ 0.5% FBS and ~~5 μ l~~ 35S cysteine.

Each well received 200 μ l. Pls were incubated 4 H @ 28°C.

Made up 4 ml of Grace's dy
+ 20 μ l FBS
+ 100 μ l of STOCK ?

HB 4°C

IP's

200 μ l of LB (receipt from George Ludwig) was added to both the media & the cells.

NET lysis buffer

4.38g NaCl

25 ml 1M TRIS, pH 8.0

5 ml 0.5M EDTA, pH 8.0

500 μ l NP40

~~5 ml~~ 10% DOC

QS to 500 ml w/ H_2O

Immediately Prior to use add:

1 ml of Cocktail Inhibitors / 100 ml of LB

200 μ l of PMSF / 100 ml of LB

{ George's
crisper
dissolves
4°C

WASH buffer

4.38g NaCl

25 ml 1M TRIS, pH 8.0

5 ml 0.5M EDTA, pH 8.0

250 μ l NP40

2.5 ml 10% DOC

QS to 500 ml w/ H_2O

Add inhibitors as in NET lysis buffer.

1 - VEE-NC 3 SINGP 5 CCHF

2 - VEE-NC 4 SINGP 6 CCHF

8 VEE-GP 7 SINGP

9 VEE-GP 10 SINGP

11 VEE-GP 15 SINGP

12 VEE-GP 18 ~~SINGP~~

13 VEE-GP

14 VEE-GP

~~15~~ VEE-GP

~~16~~ VEE-GP

V-Control = VEE

Added 25 μ l of undiluted HNAF to respective tubes.

Inc 4°C ON in end-to-end rotator.

Washed 3X, Inc 1H at 4°C, Wash 3X

Added 250 μ l of protein A-Sepharose 4B beads, Inc 1H @ 4°C

Added 150 μ l LB w/ME.

2093	Gel#	Lane#	Sub#		
	1	1	¹⁴ C - from George		
		2	¹⁴ C LH		
		3	VEE-HMAE-IP of 26 JAN 93		
		4	1 VEE NC		
		5	2 VEE NC		
		6	8 VEE SP-2 (48H)	E3, E2	
		7	9 VEE SP 6 (48H)	E3, E2, 6K, E1	
		8	11 VEE SP 2 (5D)	E3, E2 (56)	
		9	12 VEE SP 6 (5D)	E3, E2, 6K, E1 (56, 50)	
		10	13 VEE SP 2 (8D)	E3, E2 (56)	
		11	14 VEE SP 6 (8D)	E3, E2, 6K, E1 (56, 50)	
		12	16	FA SLIDE MEDIA	
		13	17		
		14	U		
		15	SB		
2		1	SB		
		2	¹⁴ C - George		
		3	¹⁴ C - LH 24		
		4	VEE-HMAE-IP of 26 JAN 93		
		5	3 SIN CP		
		6	4 SIN CP		
		7	7 SIN#1 (48H)	E3, E2	
		8	10 SIN#1 (5D)		
		9	15 SIN#1 (PD)		
		10	18 FA SLIDE MEDIA		
		11	5 CCHP-NC-HIS		
		12	6 CCHP-NC-HIS		
		13	¹⁴ C - George		
		14	¹⁴ C - LH 24		
		15	SB		

✓ Need Rocking
 1982 118, 269-6
 1985 347-351

VEE
 E2=1
 E1=3
 PE2=1
 E3=1

3- ¹⁴C markers = 40ul stock + 120ul SB/ME

IP - 5 FEB 93

ON: 5 FEB

REF: 8 FEB TA

8 Feb
Label
Chase

9:00 - 3:30
~~W/~~

W/ 4 ml of 100X cysteine

IP's of VEE + SIN Baculovirus recombinants on 24-well plds
set up 2 Feb 93.
Stained cells 1H in Grace's media + 0.5% FBS at 28°C
Added 100 µl of ³⁵S-cysteine to 4 ml Grace's media +
0.5% FBS.
Added NET buffer + INH (200 µl) placed on ice for ~20
min ^{after transferring media to Eppendorf tube}. Transferred lysate to 1.5 ml Eppendorf tube.
Stored -70°C.
Thawed cell lysates of 5 Feb 93. Added 25 µl of
HMAF. Inc @ 4°C for 2 H.
Added 250 µl of Protein A beads. Rotated 1 H @ 4°C.
Washed 3X. 1 H incubation on rotator @ 4°C.
Washed 3X. Added 150 µl SB + 2ME. Added
40 µl to SDS-PAGE gel.
Stained cells 1H in Grace's media + 0.5% @ 28°C -
(889 cells infected 2 Feb 93 with VEE + SIN
Baculovirus recombinants)
Added 100 µl of ³⁵S-cysteine to 4 ml Grace's media +
0.5% FBS.
Added 4 µl of 100X cold cysteine (from George
Sidwig) to chase.
Added 200 µl of NET LB w/ INH. Inc 10 min
at ~~4°C~~ 4°C.
Added 25 µl of HMAF. Inc on rotator ON @ 4°C.
Added 6 ml of NET lysis buffer + inhibitors to 0.75 g
Protein A-Sepharose beads. Inc 4°C on end-to-end
rotator for 10 min.
Added 250 µl of suspended beads to each of
IP's set up 8 Feb 93

Feb 93 Janett

- | | |
|----|------------------------------|
| 1 | SB |
| 2 | 14C LH24 |
| 3 | VEE-HMAF |
| 4 | 13B2-1-1 } IP's of 26 JAN 93 |
| 5 | 1 VEE NC |
| 6 | 2 VEE NC |
| 7 | 8 VEE SP-2 (48H) |
| 8 | 9 VEE SP-6 (48H) |
| 9 | 11 VEE SP-2 (5D) |
| 10 | 12 VEE SP-6 (5D) |
| 11 | 13 VEE SP-2 (8D) |
| 12 | 14 VEE SP-6 (8D) |
| 13 | 16 } FA SLIDE MEDIA |
| 14 | 17 } |
| 15 | U |

2

- | | |
|----|-------------------|
| 1 | SB |
| 2 | 14C LH24 |
| 3 | VEE-HMAF |
| 4 | 13B2-1-1 |
| 5 | 3 SINGP |
| 6 | 4 SINGP |
| 7 | 7 SIN#1 (48H) |
| 8 | 10 SIN#1 (5D) |
| 9 | 15 SIN#1 (8D) |
| 10 | 18 FA SLIDE MEDIA |
| 11 | 5 CCHF-NC-HIS |
| 12 | 6 CCHF-NC-HIS |
| 13 | 14C |
| 14 | SB |
| 15 | |

10/25/17
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 12/31/17

VEE-SIN AACULD IP's
10 FEB 93 CL #2
ON: 10 FEB 93
OFF: 11 FEB 93 7:15 AM

VEE-SIA PAULO IP'S 4-2

VEE-SIA PAULO IP'S 03
10 FEB 91 CL 41

0 N:11 FEB 91 7:15P

CA:16 FEB 3:00P

12 Feb 93
Slide

[FA]

Well

#	1	2	3	4	← Top row
1	V	V	S	V	
2	V	S	S	V	
3	~~V~~	V	V	~~S~~	
4	S	V	V	V	
5	V	S	U	U	

Media = SS-900

+ GENT
+ ~~PUT~~ FUN
+ 1% L-Glut

12-well plates

Have 8.6×10^5 cells/ml

Samples:

VEE SP2 - Original transfection media	- 5D	11
	- 8D	13
VEE SP6 - Original transfection media	- 5D	12
	- 8D	14
VEE SP2 - Media from IP study	- 3D	11
	- 6D	13
VEE SP6 - Media from IP study	- 3D	12
	- 6D	14
SIN1 - Original transfection media	- 5D	10
	- 8D	15
SIN - Media from IP study	- 3D	10
	- 6D	15

Seed 5×10^5 SS9 cells/well. Total volume of the wells should not exceed 3 ml.

Set up 3 plts = 1.8×10^5 cells / 12 well plts
 = 1.8×10^6 cells for 3 plts.
 Add 20.9 ml cells to 87 ml media.

LAB-TEX

Seed 0.5×10^6 cells/well. = 4×10^6 cells/slide
 Need 3 slides

3.2 ml/slide

4.7 ml of cells + 4.9 ml media.

Add 200 μ l of undiluted inoculum to each of the 12 wells for the 12 well plts. After 1H add 800 μ l media
 Add 100 μ l of undiluted inoculum to each of LAB-TEX wells.

Inc @ 28°C.

1	2	3	4
VEE SP2 5D	VEE SP6 5D	SIN1 5D	VEE SP2 8D
5 VEE SP6 8D	6 SIN1 8D	7 10 5FEB	8 11 5FEB
9 12 5FEB	10 13 5FEB	11 14 5FEB	12 15 5FEB

# 2	1	2	3	4
	10 8 FEB	11 8 FEB	12 8 FEB	13 8 FEB
	5	6	7	8
	14 8 FEB	15 8 FEB	VEE SP2 5D	VEE 5D
	9 SIN1 5D	10 VEE SP2 8D	11 VEE SP6 8D	12 SIN1 8D

#3	1	2	3
	10	11	12
	5 FEB	5 FEB	5 FEB
	5	6	7
pb	14	15	10
	5 FEB	5 FEB	5 FEB
	9	10	11
	12	13	14
	8 FEB	8 FEB	8 FEB

X gal 20mg/ml in DMSO
150µg/ml

50x

$$2.5 \cdot 400 = 250x \\ x = 4 \text{ ml}$$

$$50 \cdot 0.05 = 50x$$

50mg/ml

$$50x = 9.60 \\ = 10.8 \\ 2.2$$

150µg / ml
9000 µg in 60 ml
9 ~~mg~~ ~~in 60 ml~~

3

Take 12 samples to assay

- 1) 11 = VEE SP2 from Original transfusion media - 5D
- 2) 13 = " " - 8D
- 3) 12 = VEE SP6 " - 5D
- 4) 14 = " " - 8D
- 5) 11 = VEE SP2 from media from IP's - 3D
- 6) 13 = " " - 6D
- 7) 12 = VEE 6 from media from IP's - 3D
- 8) 14 " " 6D
- 9) 10-SIN1 - Original transfusion media - 5D
- 10) 15 " " - 8D
- 11) 10-SIN1 Media from IP's - 3D
- 12) " " - 6D

Will set up in duplicate: 1 set containing X-gal
" " Blue-gal

Will do undiluted.

1. Add 3.75 g of baculovirus agarose / 150 ml of Millipore H₂O Autoclave to dissolve and sterilize the solution. Inc @ 5 until needed. 5 ml of this solution will be used per 100 mm plate.

2. Add 50 μ g/ml gentamicin and 2.5 μ g/ml amphotericin to SS 900 media and 1% L-glutamine. Make up 400 ml of this media. = COMPLETE MEDIA

Gentamycin stock = 50 mg/ml

Luencyone = 250 ug / ml

3. Seed SF9 cells at a density of 5×10^6 cells/100 mm pl in complete media. (5 ml/pl)

Used 120×10^6 cells total in 120 ml of complete m

4. For each 100mm plate, place 5 ml of S/S 908 complete media for wetting surface.

5. None.

P16 : 9.2×10^5 cells/ml

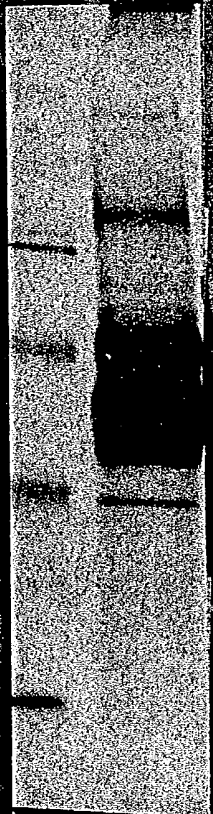
P14 : 3.4×10^5 cells / ml.

893 Set up IP's of media from SF9 cells infected w/
30P VEE SP-2, VEE SP-6, SIN1 of 5 FEB 93. Have 2
sets of IP's corresponding to 2 days harvest: 5D + 8D.
Added 50 μ l of NET buffer + inhibitors to the 200 μ l
of media. Added 20 μ l of SIN-HNAF or VEE-HMA
to respective tube. Inc 4°C ON or end to end
rotator.

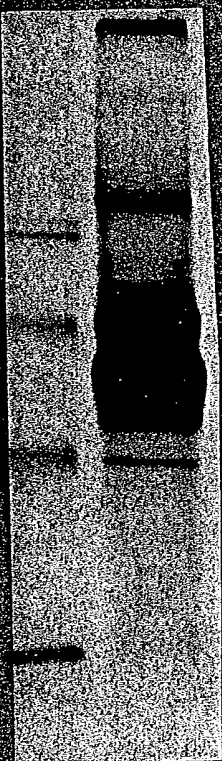
893 Added 250 μ l of Protein A-Sepharose to each tube
15A Inc 1H @ 4°C on end-to-end rotator.

<u>Gel #</u>	<u>Lane #</u>	<u>Tube</u>
1	1	SB
	2	SB
	3	14C
	4	13C - 1-1
	5	11
	6	12
	7	13
	8	14
	9	11
	10	12
	11	13
	12	14
	13	~~SB~~ Uninfected
	14	SB
	15	SB
2	1	SB
	2	SB
	3	14C
	4	13C - 1-2
	5	10
	6	15
	7	10
	8	15
	9	14C
	10	SB
	11	
	12	
	13	
	14	

EXPRESSIVE VERB



VEE SIN MACULO IT'S
DEB AEOIA
00:17 FEB 8:45 2



VEE SIN MACULO 3/3/41

STE:
 Feb: Presidents
 day - Holiday
 look off

93 Have 12-well plates to radiolabel set up on 9 Feb 93
 30A Serum 1H in Grace's media + 0.5% FBS @ 28°C.
 Removed media from cells and stored @ 4°C.
 35S cysteine. Add 100 µl STOCK (10 µCi/µl) to 4 ml of
 00A media (25 µCi/ml). Add ~~200~~ ²⁰⁰ µl to each well.
 'A added additional 200 µl of Grace's w/o cysteine + 0.5% FBS

> P Chased by adding 4 µl of cold cysteine to each well.
 4:30P Lysed cells in "traditional" LB
 993 Have 1 set up of 12-well plates that had been infected
 9 Feb 93. Media was removed, stored at 4°C.
 The cells were discarded.
 Added 10 µl of SIN-HMAF (undiluted) to 250 µl
 of Protein A-Sepharose beads. Placed at 4°C
 on end-to-end rotator. Also added 10 µl of VEE-HMAF
 to cell lysate of VEE infected BHK cells for (+) control
 2093 Added 200 µl of either lysate or media from the
 ed) 12-well plates (all SIN). Inc for 4H at 4°C
 on end-to-end rotator.

Washed 3x with "traditional" LB w/ inhibitors. Placed
 on end-to-end rotator @ 4°C for 1H. Washed
 3x with 50 + 200 µl. Added 3 µl
 Placed on 12-well acrylamide gel.
 200 µl in 12-well gel. Placed on
 end-to-end rotator for 13.

Lane	Sample
1	5B
2	5B
3	20
4	5B
5	30
6	40
7	5B
8	20
9	5B
10	5B
11	5B
12	5B

Lane	Sample
1	5B
2	5B
3	20
4	VEE-HMAF
5	SIN-50
6	20 80
7	10 50 80
8	15 85
9	20 80
10	15 85
11	20
12	20

- Feb 93 Need DNA for template for making more PCR inserts.
 Have 5 glycerol stocks from Nanosyllonics (-70°C).
 Transferred some of glycerol stocks to
 YT/AMP plates for isolation. Inc ON @ 37°C
 Feb 93 Transferred 1 colony to 2 ml YT/AMP broth. Inc
 37°C ON.
 Feb 93 No minipreps on all ON cultures.

42-384
 10 FEB 93

1st ROUND PCR

10 FEB 93

all MP's are done in duplicate.

EXPECTED								
DEPTH	500	3,914						
WA	2C	MP#4	MP#5	MP#6	MP#7	MP#8	MP#11	MP#12
WARD PR	PR#1	VC-F1						
ORSE PR	PR#2	VA-R3						
20	70.5	76.6						
TEMPERATURE	1	0.5						
WARD PR	5	1.2						
ORSE PR	5	2.7						
Rxn	18	18						
BUFFER	1							
ADNTF								
pot	0.5	0.5						
infall	100	100						
Program Step cycle: 1 min @ 95°C 30 sec @ 52°C 3 min @ 72°C Delay cycle: 10 min @ 72°C Soak cycle: 4°C								

11 FEB 93

VEE SP-7

VEE PRIMERS:

- 22 V26-F1: ^{HindIII} 5' >GGT CTA AGC TTG ^{Kozak Seq} CCG CCA CCA TGT TCC CGT TCC AGC CAA TG<3' ³¹
- 23 V26-R1: ^{SplI} 5' >GGT ACT CCG TAC ^{3' END 265} GGA ATA TTA AAA ACA AAA TCC G<3' ³⁸⁹¹
- 24 VC-F1: ^{BamHI} 5' >GGT CTA GGA TCC ^{Kozak Seq} GCC GCC ACC ATG TTC CCG TTC CAG CCA ATG<3' ³¹
- 25 VA-R3: ^{HindIII} 5' >GGT ACT CAA AGC TTG ^{3' END 265} AAT ATT AAA AAC AAA ATC CG<3' ³⁸⁹¹

V26-F1 } Synthesized for Bill Pratt - Designed for
 V26-R1 } producing PCR products of VEE-26S with
 5' HindIII and 3' SplI sites

- 394 Synthesis Setup Listing - (Version 1.01)

Column 3

3:16:33P , 2/10/93

Run ID :
 Cycle : Cyc02 user
 End Proc: End CESS (DMT = On)
 Sequence: VCF1

Total bases = 42
 A= 7, G= 10, C= 16, T= 9, S= 0, B= 0, 7= 0, 8= 0
 (mixed bases= 0)

MW: 13656.4

5' > GGT CTA GGA TCC GCC GCC ACC ATG TTC CCG TTC CAG CCA

ATG <3'

$$E_m = A(14,000) + C(7000) + G(12,000) + T(9600) \\
+ 7(14,000) + 16(7000) + 10(12,000) + 9(9600) \\
112000 + 112000 + 120,000 + 86,400 = 430,400$$

$$\frac{2434 \times 101}{30400} = \frac{24.5834}{430400} = 0.000057117 \text{ molarity} = 57.127 \text{ pmol/ul} \\
\frac{100 \text{ pmol/ul}}{57.117} = 1.75 \text{ ul per rxn}$$

DUAL λ 01

Abs

Factor= 1.000

Read average = 01

SP	260.0	280.0	XA/XB	SW	260.0	280.0	XA/XB
1	-0.0004	-0.0006	.5616	H ₂ O			
2	0.1581	0.0873	1.811	VAL3			
3	0.1503	0.0880	1.707	V26R1			
4	0.2434	0.1933	1.259	VCE1			
5	0.1256	0.0981	1.279	V26F1			

λ	ABS	SOURCE	08:36	CELL
260.0	-0.0002	UV	02/18/93	1

93 Transferred blue plaques from the plaque assays set up 26 Jan 93. (p 45 of this notebook)

		X gal	Bluegal
WT	10 ⁻¹	0	0
	10 ⁻²	0	0
pBBIII	10 ⁻¹	100+	10 ⁻¹ colonies are present but more faint than the Xgal
	10 ⁻²	11	10 ⁻² "
VEESP2	10 ⁻¹	9	10 ⁻¹ 5
	10 ⁻²	2 - Has fungus	10 ⁻² 0
VEESP6	10 ⁻¹	31	10 ⁻¹ 11
	10 ⁻²	10	10 ⁻² 9
SIN1	10 ⁻¹	25	10 ⁻¹ ND
	10 ⁻²	9	10 ⁻² 3

Set up 8 - 12 well plates = 96 wells with 5 x 10⁵ cells/well

Need 48 x 10⁶ cells

Have 0.61 x 10⁶ cells/ml

79 ml of cells + 113 ml media (Add 2 ml of media/well)

Media = SF900

GENT

FUNG

1% L-CLUT

Removed plaque with plastic pipettors. Transferred agarose plug directly into media of well.

#		
1-9	VEESP2	10 ⁻¹ Xgal
10-14	"	10 ⁻¹ Bluegal
15-45	VEESP6	10 ⁻¹ Xgal
46-56	VEESP6	10 ⁻¹ Bluegal
57-81	SIN1	10 ⁻¹ Xgal
82-85	SIN1	10 ⁻² Bluegal
86-89	pBBIII	10 ⁻¹ Xgal
90-96	Uninfected	

93 PCR Analysis of Recombinant Virus (Ref: Invitrogen Maxam Instruction Manual Version 1.5.6. p26)

The forward and reverse PCR primers provided by Invitrogen (Cat# N610-04) are chemically synthesized oligomers which are complementary to the polyhedrin loci. The polyhedrin gene spans base +1 to +738. As shown below the forward and reverse primers have been designed to flank

Top 2, for 1600 marker

Bolton 13, 14, 15, 16 ^{slow 1600} marker

These primers are compatible w/ all of Invetrogen's polyhedrin promoter based baculovirus transfer vectors which, when amplified without inserts, give the following fragment sizes following agarose gel electrophoresis.
 Nox-Tus⁺ Vector, pBlueBac⁺ 620bp.

As the size of the foreign gene insert is known, the size of the PCR product expected can easily be determined and wild type contamination detected.

Used media from the 12-well plates set up on 17 Feb 93. There was fungus in many. these were discarded. The following were analyzed for recombinant virus.

PP#	VIRUS INSERT	PCR RESULTS	PP#	VIRUS INSERT	PCR RESULTS
1	VEE-SP2 (1492bp)		59	S101	
2	"		61	"	
3	"		62	"	
4	"		64	"	
6	"		68	"	
7	"		71	"	
10	"		74	"	
11	"		75	"	
12	"		76	"	
13	"		77	"	
14	"		80	"	
15	VEE-SP6 2,889bp		ND81	"	
18	"		ND83	"	
19	"		ND84	"	
22	"		ND85	"	
23	"		86	pBBIII	
24	"		ND90	uninfected	
28	"		49 samples		
32	"				
33	"				
38	"				
41	"				
42	"				
44	"				
46	"				
49	"				
50	"				
53	"				
54	"				

1. Pipette the media in the microtiter plate well up & down to dislodge the cells then remove 0.75 ml of media and place in a sterile Eppendorf tube. This was used to carry out PCR analysis of the putative recombinant virus. The remaining media + cells ^(~1.5 ml) was transferred to another Eppendorf tube and stored at -70°C .

2. Pellet the cell debris by centrifugation at 5000 rpm for 3 min in Sorvall.

3. Transfer the supernatant to a fresh tube and add 0.75 ml of a solution of 20% polyethylene glycol (PEG) in 1 M NaCl. (PEG = Sigma Cat # P-5413, MW=8000).

For 42 ml of solution used 10.5 ml of 4 M NaCl and 8.4 g of PEG. ^{3.5 ml H_2O} ^{1.5 ml initially to make 42 ml QS.} Invert twice to mix and allow to stand at RT for 30 min.

4. Centrifuge at 4000 rpm for 10 min at RT. Remove all media from the pellet. An additional quick spin may be required to remove trace amounts of media. The pellet may not be visible at this point.

5. To the pellet add 100 μl of sterile water. Carefully wash the sides of the tubes to ensure that all of the viral particles are resuspended.

6. Add 5 μl of Proteinase K (20 mg/ml) (Amber Cat #). Incubate at 50°C for 1 hr.

7. Extract with an equal volume of phenol/chloroform (1:1). Centrifuge in an eppendorf centrifuge for 5 min. Transfer the upper aqueous phase to a fresh sterile tube.

8. Precipitate the DNA by adding $1/10$ volume 3M NaOAc pH 5.2, 5 μl glycogen (didn't add) and 2 volumes of 100% EtOH. Incubate at -20°C for at least 20 min. Incubated ON.

9. Centrifuge at 14,000 rpm for 15 min @ 4°C . Wash pellet with 80% EtOH. ~~Inc - 70°C~~ Spin 14,000 rpm for 5 min to remove all traces of EtOH. Dry DNA on edge of hood - 20 min.

10. Resuspend the pellet in 10 μl of sterile water. The DNA is now ready for analysis by PCR.

PCR
Add 20 μl of sterile water to primers. Concentration of primers - 2 μl of each primer lyophilized in 10 mM Tris-HCl

DUAL λ		01	Abs	Factor= 1.000			
		Read average = 01					
S#	260.0	280.0	$\lambda A/\lambda B$	S#	260.0	280.0	$\lambda A/\lambda B$
<i>out in 500ul H₂O</i>							
1	0.0016	0.0011	1.360				
2	0.7288	0.4111	1.773 f1				
3	0.7826	0.4650	1.683 l1				

λ	ABS	SOURCE	15:45	CELL
260.0	0.0027	UV/Vis	02/23/93	1

- 394 Synthesis Setup Listing - (Version 1.01)

Column 2

4:08:52P , 6/22/92

Run ID :

Cycle : Cyc02 user

End Proc: End CESS (DMT = On)

Sequence: HISRI

Total bases = 20

A= 9, G= 3, C= 6, T= 2, S= 0, B= 0, 7= 0, 8= 0
(mixed bases= 0)

MW: 6629.0

5' > CAA CAA CGC ACA GAA TCT AG <3'

$$\begin{aligned}
 E_{260} &= A(16000) + C(7000) + G(12000) + T(9600) \\
 &= 9(16000) + 6(7000) + 3(12000) + 2(9600) \\
 &= 144,000 + 42,000 + 36,000 + 19,200
 \end{aligned}$$

$$E_m = 241,200$$

$$\text{Molarity} = \frac{OD_{260} \cdot \text{dil factor}}{E_m}$$

$$= \frac{0.7826 \cdot 51}{241,200}$$

$$= \frac{39.9126}{241,200}$$

$$= 0.000165475$$

$$\text{Molarity} = 165.5 \text{ pmol/ul}$$

Need 50 pmol for PCR

PCR screening of Recombinants VEE SP 2, VEE SP 6, SIA GP.

[NOTE!] PP#s are not inclusive

A	LC	VEE SP 2 PP# 1-14	VEE SP 6 PP# 15-55	SIA GP PP# 57-81	PBB III PP# 86	UNINF. PP# 90
PR	Pr# 1	~~10VITROGEN CAT# N670-04 LOT # 110514~~ ~~FOR PR~~ HIS F1				
PR	Pr# 2	REVERSE PR. HIS R1				
bp)	500	1,492	2,889	1,492	620	0
ut)	35.2					
PLATE	0.5	5 ul	5 ul	5 ul	5 ul	5 ul
PR	2.5	0.4				
PR	2.5	0.3				
BUFF	9 ul	MM(I) 9	9	9	9	9
NTP						
of SU)	0.3					
out	50 ul					
Program: Master Mix (I): 1 cycle: 1 min 94°C 10X RXN buffer = 5 x 50 = 250 2 min 55°C dATP 1 x 50 = 50 3 min 72°C dCTP 1 x 50 = 50 7 min 72°C dGTP 1 x 50 = 50 40°C dTTP 1 x 50 = 50						

VEE SP 2

VEE SP 6

3
21
10
5
8

Maxiprep: VEE SP 2 (6-5) 8.19 ug/ut
 VEE SP 3 0.59 ug/ut
 VEE SP 6 (1-1) 5.3 ug/ut
 SIN #1 4.6 ug/ut
 pBBIII (Mike Parker) 1.1 ug/ut
 #77 1.1 ug/ut

0.4 ut = 3 ug (+)
 5 ut = 3 ug
 0.6 ut = 3 ug
 0.7 ut = 3 ug
 2.7 ut = 3 ug

Maxiprep
 264 ut 92 Molestock
 3426

} V-1-2
 } C-29-2

1.96 ug/ut
 1.53 ug/ut

1.53 ut = 3 ug
 1.97 ut = 3 ug

Have 1.35×10^6 cells / mt. No dead cells seen.
Need 2×10^6 cells / 60mm plate.

Need 2×10^6 cells / 60 mm plate.
 Have 8 transformations = 16×10^6 cells total - actually by
 use 11.9 ml/cells + 4.1 ml SF900 media (complete) ^{uninf} _{control}
 Use 2 ml of SF900 media (complete / 60 mm well) = 16 ml
 Media : SF900

sent 50 ut in 50 mt

Fung 500 ml in 50 ml

1% L-GLUT 500 μ l in 50 ml

0.5% FBS 250 ul in 50 ml

Cationic Liposome Mediated Transfection of SF9 cells

1. For each 60 mm plate to be transfected set up the following transfection mix: Pipette 1 ml of Grace's media (Cat # B823-01) containing no supplements into a 1.5 ml sterile eppendorf. Add 1 μ g of linear A α MNV DNA (Cat # B825-03) and 3 μ g of plasmid DNA (the transfer vector containing the gene of interest). Thoroughly mix the Cationic Liposome solution then add 20 μ l to the DNA/Grace's media solution. Vortex vigorously.
2. Incubate at RT for 15 min.

2. Incubate at RT for 15 min.

3. During this incubation period remove the media from the plates and replace w/ 2 ml of Grace's media w/ 6 supplements added. Allow the cell to sit until the incubation in Step 2 is with 2 min of completion. This should be ~ 10 min.

4. Remove all of the *Thaps medea* from the cells and add the 1 ml transfection mix dropwise to the 60 mm plate.

8. Incubate plates on a slowly rocking platform at RT for 4 H. Use 2.5 setting on Bellco side/side rocking platform.

6. Following this incubation period add an additional 1 ml of complete ~~FF~~ Sf900 media to each 60 mm plate and incubate at 27°C in a humidified environment for 48 h.

Remove media from the transfected plates and store at 4°C until needed. This media is the virus stock which will be used in the identification of recombinant virus by plaque assay. Replace the media with 3 ml of complete DMEM.

$$\begin{array}{r} 37 \\ 36 \\ \hline 7400 \text{ ug} \\ 7400 \text{ ug} \end{array}$$

148 ml Benzoin 185 ml
TNM

$$\text{Stock Gent} = 50 \text{ mg/ml}$$

$$10 \cdot 300 = 50,000 \times$$

$$\text{Stock fung} = \frac{250}{\cancel{250}} \text{ ug/ml}$$

$$250 \times = 2.5 \cdot 300$$

$$\times = 3$$

3 ul per ml 15 ul

STOCK: 50 mg/ml
Need 300 ug / ~~ml~~ 15 ml

$$50,000 \cdot \times =$$

93 Plaque Assay *get show signs of infection*

Media: TNM-FH - Make up 300 ml
+10% FBS - 30 ml

10 ug/ml gentamicin - 60 ul / 300 ml
2.5 ug/ml Fungizone - 3 ml / 300 ml

1. Prepare 10^{-1} fold dilutions of virus inoculum (1:10, 1:100)
from media harvested from 48-H transfection set up
23 Feb 93.

2. Seed SF9 cells at a density of 5×10^6 cells / 100 mm plate
in complete media.

Have 1×10^6 cells/ml

Set up 20 plates = $20 \times 5 \times 10^6$ cells / 100 mm plate =
~~100~~ 10^6 cells

Have 2 SF9 cell culture

1 = 1×10^6 cells/ml

Unlabeled: 1.13×10^6 cells/ml (clumps present)

Need 5 ml media + cells / plate = 110 ml

Need 100×10^6 cells in 110 ml media

Only have ~ 75 ml of cells of 1×10^6 cells/ml

75 ml $\pm$ enough for 15 plates (75 ml media)

Use ~~20~~ ml of 1.13×10^6 cells/ml. Need 35×10^6 cells

Use ~~31~~ ml of cells + 4 ml of media.

Need 1 ml of inoculum / plate 200

Make up ~~25~~ ml of 1:10 = ~~20~~ ul of undil + ~~2~~ ml of media

1:100 = 200 ul of 1:10 + 1.8 ml media

Added ~~200~~ ul of 50 mg/ml Blugol stock solution to

100 ~~50~~ ml TNM-FH complete media. Placed 5 ml of
media into each of 20 tubes

Added mixed media (w/ penicillin) by pipetting slowly into 1 section of the
plate.

Inoculums: 48H harvest of:

VEE SP2 10^{-1} , 10^{-2}

VEE SP3 10^{-1} , 10^{-2}

VEE SP6 10^{-1} , 10^{-2}

SIN #1 10^{-1} , 10^{-2}

pBBIII

WT

V-1-2

C-29-2

4 MAR

No blue plaques

"

"

"

"

"

2 blue plaques

No blue plaques

8 MAR

No blue f

"

"

1 blue plag

1 blue plag

No blue fl

"

"

93 Plaque Assay

Media: TNM-FH - Make up 300 ml (Complete)
+10% FBS - 30 ml

10 μ g/ml gentamicin = 60 μ l / 300 ml

2.5 μ g/ml fungizone = 3 ml / 300 ml

1. Seed SF9 cells at a density of 5×10^6 cells / 100 mm pl.

Have 2 flasks of ~ 75 ml each P22 on 3MAR

#1 $\frac{240}{5} \cdot 1.5 \times 10^4 = 0.72 \times 10^6$ cells/ml

#2 $\frac{290}{5} \cdot 1.5 \times 10^4 = 0.87 \times 10^6$ cells/ml

Will set up 20 plates = 100×10^6 cells total

Mix the 2 flasks together = 0.795×10^6 cells/ml

~~Add 25.6 ml to~~ Add 6.3 ml to each plt.

Allow to settle for 5 min. Place on end to end rocker (Setting = ~~30~~) for 25 min. Place on bench top for 30 min.

2. Dilute virus in TNM-FH (complete)

Need 1 ml of inoculum / plt.

Make up 2.5 of 1:10 = 200 μ l of STOCK + 1.8 ml of me

1:10 = 200 μ l of 1:10 + 1.8 ml media

Have 4D Harvest of

VEESP2

10^{-1}

10^{-2}

VEESP3

"

"

VEESP6

"

"

SIN#1

"

"

pBBIII

"

"

WT

"

"

V-1-2

"

"

G-29-2

"

"

Control - NO DNA Audit

Cells only

93 1/2-well plate

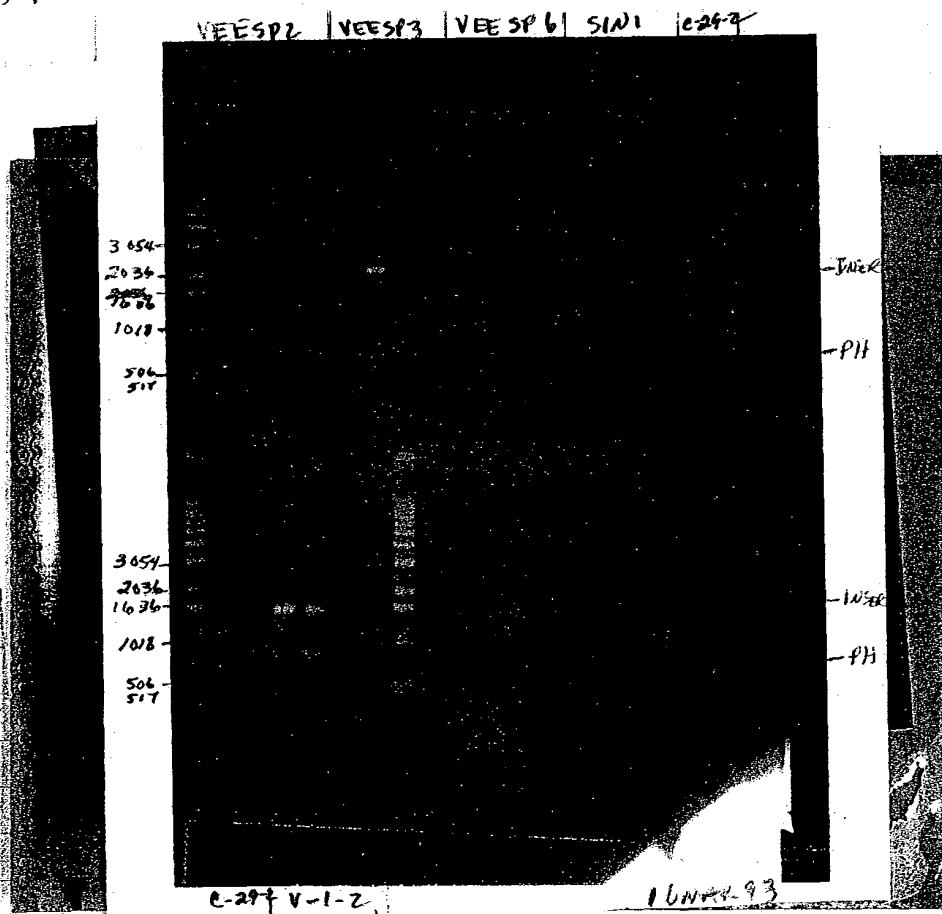
1. One-well wells of 2-12 well plate w/TPM.FH
2. Seed 5×10^5 cells in each well. Total volume should not exceed 3 ml.

Have 24 wells = 12×10^6 cells total in 12 ml med
 Have 0.795×10^6 cells/ml STOCK = 15 ml cells + 51 ml.

93 Used SOP on p93 of this notebook w/ modifications to isolate DNA for PCR screening. ^{remaining media stored} DNA stored @ -20°
~~HIS R2~~ Plaque pick #1

	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	
VEE SP 2	—	PH	—	PH	PH = Polyhedrin present
VEE SP 3	—	PH	PH	PH	
VEE SP 6	—	PH	PH	PH	
SIN 1	—	—	—	—	
C-29-2	—	—	PH	PH	
V-1-2	—	PH	PH	—	

93 Cathy Lind did PCR amplification of the isolated DNA using HIS-F1 and HIS R2 primers. (Protocol 9-PCR analysis of Recombin. Virus, p26. Maxam Instruction Manual V. 1.5.6)



Gel inserts at SP3-2 $1647bp + 620 = 2267$
 SP6-1, $1889bp + 620 = 2509bp$
 V-1-2, 1, 2 $861bp + 620 = 1481$

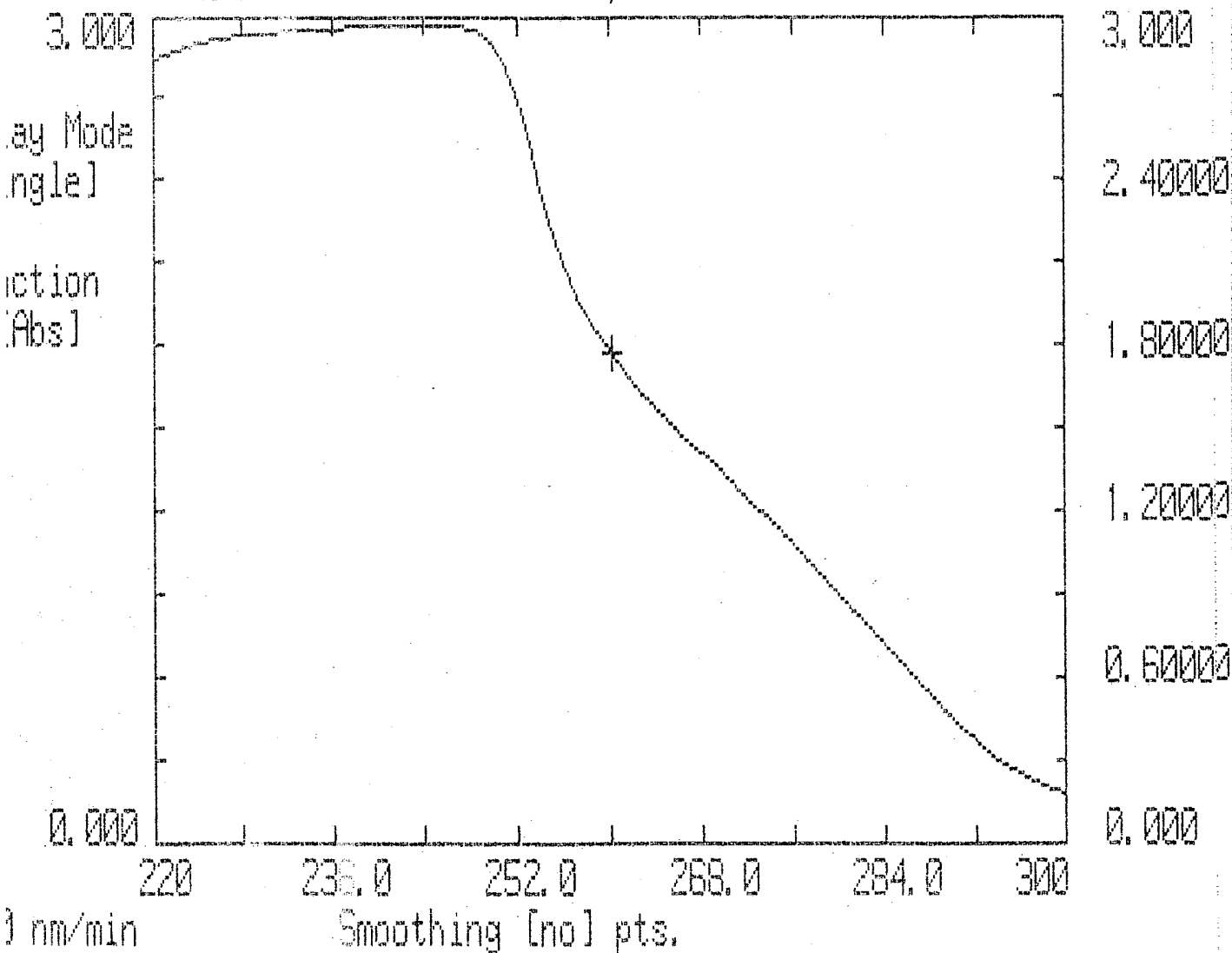
DUAL λ 01 Abs Factor=1.000
 Read average = 01

W	260.0	280.0	AA/AB	W	260.0	280.0	AA/AB
	50 μ l sample						
1	0.0001	0.0004	0.3333				
2	1.7948	0.9364	1.916	DNA PREP VCF1/VCR2 - GPC 4 MAR 93 = VEESP-1			
3	1.0430	0.5760	1.810	"	VE3F3/VAR3 " " = VEESP-5		

#1 $89.9 \mu\text{g/ml} = 0.0899 \mu\text{g}/\mu\text{l} = 4.5 \mu\text{g total sample}$
 #2 $52.15 \mu\text{g/ml} = 0.05215 \mu\text{g}/\mu\text{l} = 2.6015 \mu\text{g total sample}$

λ ABS SOURCE 09:20 CELL
 260.0 1.0478 UV/Vis 03/08/93 sgla

Scan # 01.01 VC-F1/VCR2



3 Restriction Digests of VEE SP-1, VEE SP-5

VEE SP-1 (VCR1/VCR2) = 4.5 μ g / 50 μ l (861 bp)

VEE SP-5 (VE3F3/VAR3) = 2.61 μ g / 50 μ l (3,095 bp)

PBB III (Innistragen) - 10 μ g / 80 μ l

Restriction endonuclease : 100 μ l reactions

To each tube add

10 μ l Restr 2 buffer (10X) & 712

2 μ l HIND ~~III~~ III

Inc 1.5 H @ 37°C

Add 0.1 μ l 5M NaCl

2 μ l Bam HI

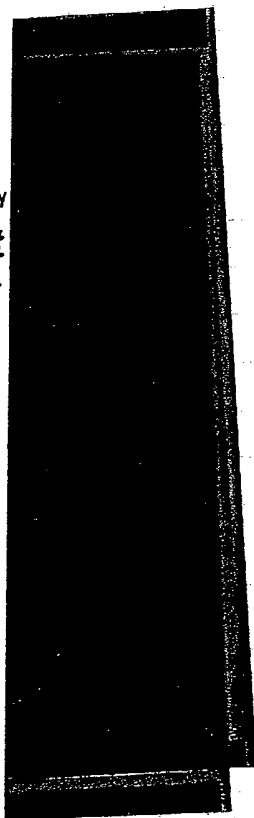
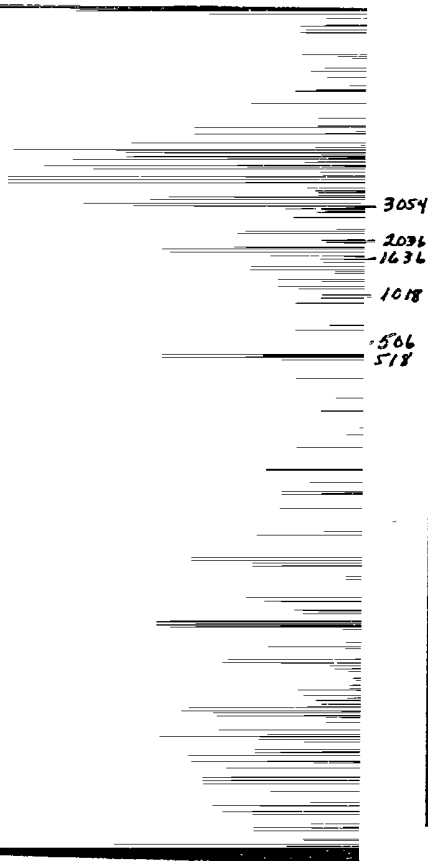
Inc 1.5 H @ 37°C

Ice 10 min.

Add 38 μ l to
VEE SP-1, VEE SP-5
tubes

VEE SP-1

VEE SP-5



14.8



PCR screening of recombinant.
19 MAR 93

DNA	7C	C-29-2	SINI	VEESPR	VEESPB	PBBTII
FORWARD PR		HISF1				
REVERSE PR		HISR1				
H ₂ O	35.2	35				
DNA TEMPLATE	0.5	5				
FORWARD PR	2.5	0.4				
REVERSE PR	2.5	0.3				
10X RN BUFF	5	9				
dNTP	1 ul ea					
Taq pol	0.3	0.3				
TV		50 ul				
Thermostabil		50 ul				
Thermocycler Program						
Step cycle: 1 min @ 94°C						
2 min @ 55°C						
3 min @ 72°C						
Defay cycle: 7 min @ 72°C						
Soak cycle: 4°C						
Super Mix						
10X RN BUFFER	5	13 = 65				
dATP	1	13 = 13				
dCTP	1	13 = 13				
dGTP	1	13 = 13				
dTTP	1	13 = 13				
HISF1	0.4	13 = 5.2				
HISR1	0.3	13 = 3.9				
H ₂ O	35	13 = 455				
Taq pol	0.3	13 = 3.9				
		13 158.5				
		45				

30 cycles

52

293

12-well plates

1. Pre-mix wells of 1 - 12 well plate w/ TNM.FH.
2. Seed 5×10^5 SS9 cells in each well. Total volume should not exceed 3 ml.

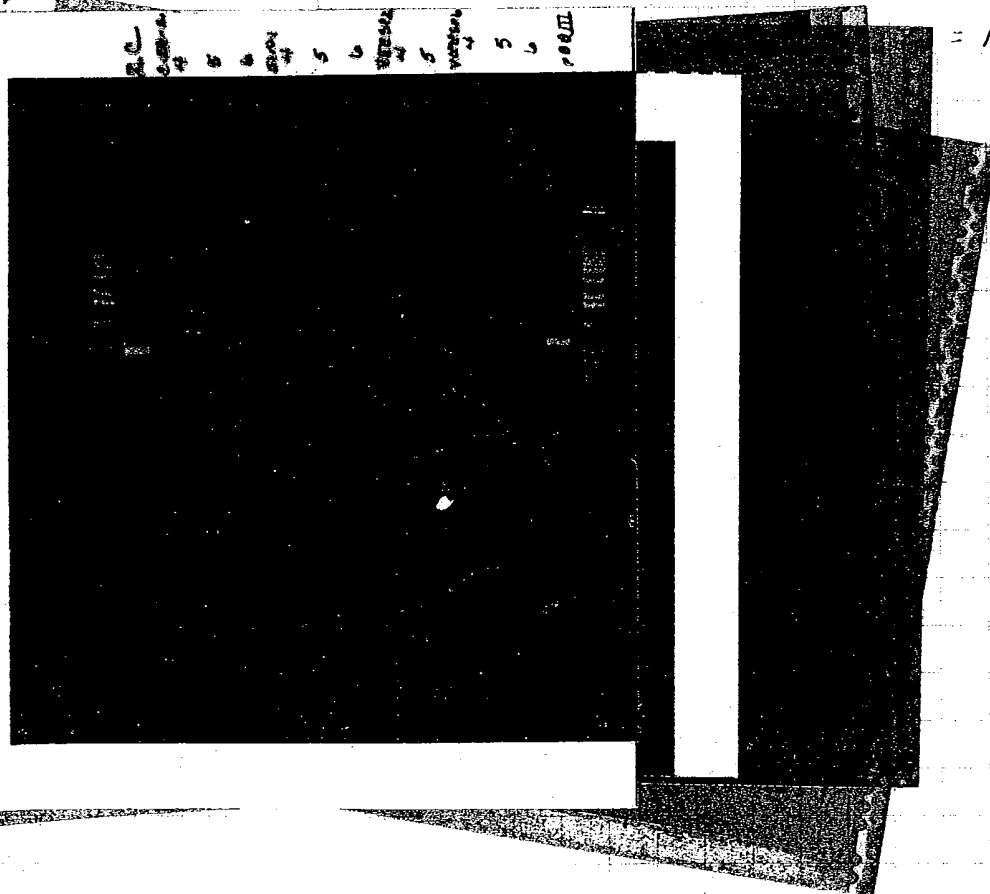
Have 12 wells = $60 \times 10^5 = 6 \times 10^6$ cells total in 36 ml

Have $\frac{290}{5} \cdot 1.5 \times 10^4 = 87 \times 10^4 = 0.87 \times 10^6$ cells/ml

Use 6.9 ml of cells + 29.1 ml of TNM.HH complete.
Picked following plaques

	Plaque Pick #			
	4	3	6	
~~C-29-2~~	~~PH~~	~~PH~~	~~PH~~	
C-29-2	PH	PH	PH	PH = Polyhedrin present
SIN1	PH	PH	Blue-PH	(-) = no PH
VEESP2	PH	PH	ND	ND = not done
VEESP6	PH	PH	(-)	
PBBIII	Blue	ND	ND	

MRB Used 50 P on p93 of this notebook. w/o modification to isolate DNA for PCR screening. DNA stored at -20°C . Stored remaining media @ -70°C . Used 150 ul to infect ^{SS9 cells on} LAB-TEK slides for FA.



FA

Have 24 samples

Set up LAB-TEK SLIDES

Need 0.5×10^6 cells / 8 well LABTEK

$400 \mu\text{t} / \text{well} = 3.2 \text{ ml} / \text{slide}$

Have 0.87×10^6 cells / ml

Need 3.5×10^6 cells / slide in ~~3~~ 22.4 ml

TNMFH (complete)

Add 4 ml of cells + 18.4 ml media

Infect w/ .150 ml of following:

VEE SP2 PP 1

" 2

" 3

" 4

VEE SP3 PP 1

" 2

" 3

" 4

VEE SP6 PP 1

" 2

" 3

" 4

✓ SIN1 PP 1

" 2

" 3

" 4

C-29-2 PP 1

" 2

" 3

" 4

✓ V-1-2 PP 1

" 2

" 3

" 4

VEE SP2 2D 10^{-1}

~~10~~ 10^{-2}

VEE SP3 2D 10^{-1}

10^{-2}

VEE SP6 2D 10^{-1}

10^{-2}

SIN1 2D 10^{-1}

10^{-2}

PBBIII 2D 10^{-1}

10^{-2}

WT 2D 10^{-1}

10^{-2}

V-1-2 2D 10^{-1}

10^{-2}

C-29-2 2D 10^{-1}

10^{-2}

VEE SP2 4D 10^{-1}

10^{-2}

VEE SP3 4D 10^{-1}

10^{-2}

VEE SP6 4D 10^{-1}

10^{-2}

SIN#1 4D 10^{-1}

10^{-2}

PBBIII 4D 10^{-1}

10^{-2}

WT 4D 10^{-1}

10^{-2}

V-1-2 4D 10^{-1}

10^{-2}

C-29-2 4D 10^{-1}

need to amplify

need to amplify

need to amplify

Restoration Requests of VEE SP-4, VEE SP7, pBBIII

DUAL λ

01

Abs

Factor = 1.000

Read average = 01

260.0 280.0 $\lambda A/\lambda B$ SH 260.0 280.0 $\lambda A/\lambda B$

Bam HI / Hind III, G-B/GC

50 μ l samples

SH	260.0	280.0	$\lambda A/\lambda B$	SH
1	0.0004	0.0004	.8672	
2	0.7923	0.4924	1.609	VEE SP1
3	0.6951	0.4312	1.612	VEE SP4
4	0.4656	0.2801	1.662	VEE SP5
5	0.4874	0.2693	1.809	VEE SP7
6	2.9900	2.8552	1.047	pBBIII

#2 39.615 μ g/ml = 0.039615 μ g/ μ l 1.981 μ g total sample

#3 34.755 μ g/ml = 0.034755 μ g/ μ l 1.74 μ g "

#4 23.28 μ g/ml = 0.02328 μ g/ μ l 1.164 μ g "

#5 24.37 μ g/ml = 0.02437 μ g/ μ l 1.2185 μ g "

#6 149.5 μ g/ml = 0.1495 μ g/ μ l * needs to be diluted
sample was still in EtOH.

λ	ABS	SOURCE	11:26	CELL
260.0	2.9838	UV/Vis	03/10/93	sqle

DUAL λ

01

Abs

Factor = 1.000

Read average = 01

SH 260.0 280.0 $\lambda A/\lambda B$ SH 260.0 280.0 $\lambda A/\lambda B$

50 μ l sample

SH	260.0	280.0	$\lambda A/\lambda B$	SH
1	0.0000	0.0003	.0000	
2	2.1146	1.1843	1.785	pBB/H/B/RIAP/ ϕ /EtOH

#2 105.73 μ g/ml = 0.10573 μ g/ μ l = 5.3 μ g/sample

λ	ABS	SOURCE	18:44	CELL
260.0	2.1047	UV/Vis	03/10/93	sqle

GIBCO BRL

LIFE TECHNOLOGIES, INC.
GAITHERSBURG, MARYLAND 20898

5224SB

Lot No. CMC105

T4 DNA Ligase

100 units; 1 U/ μ l

Store at -20°C (not frost-free)

Description:

T4 DNA Ligase can be used to join DNA fragments with staggered or blunt ends and to repair nicks in double-stranded DNA having 3'hydroxyl and 5'phosphate ends. The enzyme is isolated for *E. coli* lambda lysogen NM989.

Unit Definition:

One (Weiss) unit catalyzes the exchange of 1 nmol ^{32} Pi into [γ/β - ^{32} P]ATP in 20 minutes at 37°C.

Components:

5224SB	T4 DNA Ligase	Lot No. CMC105
Y90001	5X DNA Ligase Buffer	Lot No. CJ0104

Storage Buffer:

10 mM Tris-HCl (pH 7.5)
50 mM KCl
1 mM DTT
50% (v/v) glycerol

5X DNA Ligase Reaction Buffer:

250 mM Tris-HCl (pH7.6)
50 mM MgCl₂
5 mM ATP
5 mM DTT
25% (w/v) polyethylene glycol-8000.
Store buffer at -20°. Due to the presence of dilute ATP, the shelf life of the buffer is 6 months.

Note:

FOR OPTIMAL TRANSFORMATION, DILUTE THE LIGATION REACTION $\geq$ 5-FOLD PRIOR TO ADDING TO COMPETENT CELLS¹.

Quality Control Data: See Back Page

Effective 05/15/92

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE IN HUMANS OR ANIMALS.

93 Ligations of pBBIII and VEESPI, VEESPI4, VEESPI5, VEESPI7
 original copy in Cathy Lind's notebook.

x#	H ₂ O	3X LIGASE BUFFER	pBBIII (0.25 μ g)	VEESPI (μ g)	VEESPI4 (μ g)	VEESPI5 (μ g)	VEESPI7 (μ g)	T4 DNA LIGASE	TV (μ l)
—	6	2.3	18.9 (0.75 μ g)	—	—	—	—	3	30
1.8	3	2.3	6.4 (0.25)	—	—	—	—	1.5	15
2.7	2	2.3	2.0 (0.08)	—	—	—	—	1	10
18.2	6	0.19 (20ng)	2.1 ⁶ (0.1)	—	—	—	—	3	30
—	6	2.3	—	21.6 (0.75)	—	—	—	3	30
4	3	2.3	—	7.2 (0.25)	—	—	—	1.5	15
2.4	2	2.3	—	2.3 (0.08)	—	—	—	1	10
18.01	6	0.19 (20ng)	—	2.8 (0.1)	—	—	—	3	30
—	6	2.3	—	—	21.5 (0.5)	—	—	3	30
1	4	2.3	—	—	14.7 (0.25)	—	—	2	20
1.3	2	2.3	—	—	3.4 (0.08)	—	—	1	10
16.51	6	0.19 (20ng)	—	—	4.3 (0.1)	—	—	3	30
—	6	2.3	—	—	—	20.5 (0.5)	—	3	30
1.4	4	2.3	—	—	—	10.3 (0.25)	—	2	20
1.4	2	2.3	—	—	—	3.3 (0.08)	—	1	10
16.71	6	0.19 (20ng)	—	—	—	4.1 (0.1)	—	3	30
4.1	2	2.3	—	—	—	—	—	1	10

Inc. ON @ 16°C

93 Miniprep set up
 9-1 VEESPI
 9-2 "
 13 VEESPI7
 screened up
 set up again -
 2ml from Grid plasmid set up 13 MAR 93

Placed in 2ml LB + AMP

93 Isolated DNA from MP broths set up 11 Mar 93.
 Have additional colonies

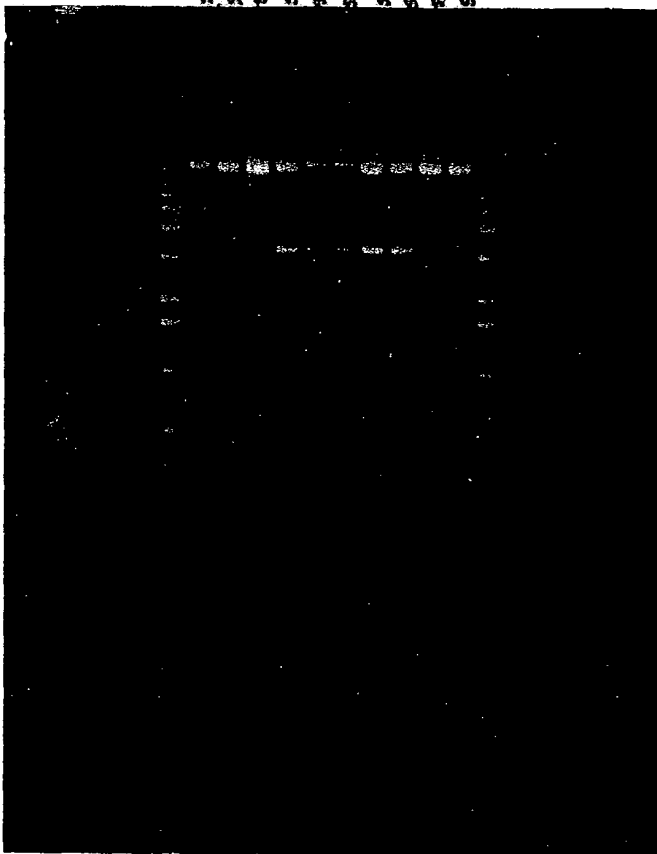
1 VEESPI
 11-1 VEESPI5
 11-2 "
~~screened up~~

93 Isolated DNA from MP broths set up 13 Mar 93
 Have additional colonies

10
 11-3, 11-4
 15
 set up 9-1, 9-2, 13

93 Restriction analyses w/ Bam HI and Hind III were done
 by Cathy Lind. See photo of gel next page.

SP7-15
SP7-14
SP7-13
SP7-12
SP7-11
SP7-10
SP7-9
SP7-8
SP7-7
SP7-6
SP7-5
SP7-4
SP7-3
SP7-2
SP7-1



4072
3054
2036
1636
1018
506
517

16 MAR 93

MAR 93 [252] (Ref. p 28 MAX BAC Instruction Manual V. 1.5.6)
 Preparing large Scale High Titer ~~Stocks~~ ^{virus stocks}

1. Inoculum: P1 viral stock harvested from the 12 well ~~plates~~ plates that were picked based on having blue plaques.
2. Seed 2 - 25 cm² flasks w/ 3×10^6 SF9 cells (p. 16 of manual)
3. Add 100 μ l of the P1 virus stocks and incubate at 27°C for 4-5 days until the cells are 90% lysed.

Inoculum: V-1-2 PP#1, PP#2 } 1 mt volume of inoculum (p. 16 of manual)

Have 1.05×10^6 cells / mt.

Have a total of 78.75×10^6 cells

Can make up ~~5~~ ²⁰ flasks if needed.

Can do all of 2D & 4D harvests of original transfections of 23 Feb 93.

Also do Make up cells in 5 mt of media = 130 mt of mix
 Mix 74 mt of cells + 56 mt of media

Inoculum:

VEE SP2 - 2D

4D

VEE SP3 - 2D

4D

VEE SP6 - 2D

4D

VEE SP3 PP#1-4

VEE SP6 PP#1-4

V-1-2 PP#1-4

SIN#1-2D

4D

SIN1-PP#6

C-29-2 - 2D

- 4D

Media Harvested 23 Feb 93 from original transfects

Media Harvested 23 Feb 93 from original transfects

Media Harvested 23 Feb 93 from original transfects

22 93 Media clarified by centrifugation at 3,800 rpm for 3 min in 15 mt tubes. Cells discarded. Media transferred to 8 - 1.5 mt Nalgene tubes and frozen at -70°C

42 93 PCR screening for baculovirus recombinants:
 750 μ l of media was PEG/NaCl precipitated, DNA was isolated by EtOH ppt after ϕ /chloroform extraction. PCR amplified w/ H1SF1/H1BR1 primers as indicated in protocol by Invitrogen MAX BAC instruction manual

CR Analysis of Recombinant virus 18 MAR 93

	2C	VEESP2	VEESP3	VEESP6	V-1-2	SIN1	C-29-2	U
PR	1415 K1							
PR	1415 R1							
	560	1492	1647	2889	861	~1500	1670	0
	35							
PLATE	5ul							
PR	0.4							
PR	0.3							
BUFF	9							
ITP								
	0.3	0.3						
V=	50ul							
vol	50ul							

cycles Program

cycle: 1 min @ 94°C
 2 min @ 55°C
 3 min @ 72°C
 cycle: 7 min @ 72°C

Master Mix (T):

10X RXN buffer
 dATP
 dCTP
 dGTP
 dITP

"Super" Mix

5X 26 = 130 forward prod. 0.4 x 26 = 10.
 1X 26 = 26 Reverse prod. 0.3 x 26 = 7.
 1X 26 = 26 M.M.I. 9 x 26 = 23.
 1X 26 = 26 Tag prod. 0.3 x 26 = 7.
 1X 26 = 26 H₂O 35 x 26 = 910

VEE SP2	VEE SP3	VEE SP6	VEE SP3	VEE SP6	V-1-2
10 40	20 40	20 40	1 2 3 4	1 2 3 4	1 2 3

Approx Results (bp)

Expected

1492 + 620 =

2112

VEE SP2

1647 + 620 =

2267

VEE SP3

2889 + 620 =

3509

VEE SP6

1647 + 620 =

2267

VEE SP3

2889 + 620 =

3509

VEE SP6

861 + 620 =

1481

V-1-2

365V
 2036
 1636
 1018
 506
 518



1600

1481

1481

8 MAR 93 Plaque assay

119

Media: TM-FH. Make up 300 ml complete media

270 ml TM-FH

30 ml FOS (10%) final conc

~~300 ml~~ Gentamicin (50 µg/ml = final conc)

2 ml fungizone (2.5 µg/ml = final conc)

1. Seed cells at a density of 5×10^4 cells/100 mm plate.

Have 27 plates to set up. Need 135×10^6 cells total

Have 2 flasks

$$P26 \# 1: \frac{382}{5} \cdot 1.5 \times 10^4 = 1.15 \times 10^6 = 86.25 \times 10^6 \text{ cells} = \text{total}$$

$$P26 \# 2: \frac{357}{5} \cdot 1.5 \times 10^4 = 1.07 \times 10^6 = 80.25 \times 10^6 \text{ cells} = \text{total}$$

$$2(2.22)$$

$$1.11 \times 10^6 \text{ cells/ml}$$

Mix the 2 flasks together = 1.11×10^6 cells/ml

Add 121.6 ml of cells + 13.4 ml of media = 1×10^6 cells

Add 5 ml of 1×10^6 to each plate.

Allow to settle for 5 min. Place on tilt bed rocker for 25 min (setting = 5.0) for 25 min. Place in hood for 30 min.

Inoculum: 1 ml of undiluted media

C-29-2 - 2D

C-29-2 - 4D

V-1-2 - PP1

" 2

" 3

" 4

SIN1 - 2D - Blue Plaque 22 MAR 93

" 4D

" PP6

VEESP 2-2D

" - 4D

VEESP 3-2D

" - 4D

" PP1

" 2

" 3

" 4

VEESP 6 - 2D

" 4D

" PP1

" 2

" 3

10X amp:

ZOWATT II
2000
2000

1412
8054
2076
162
1018
506
817

VEE SP7 = 3,914 bp
(265)

VCFI/VAR3

4072

3054

2036

1636

1018

22ND93

VEE SP4 = 3,095 bp
(OTA)
(E3, E2, 6K, E1 → 3' END)

VEE3/VAR3

2036

1636

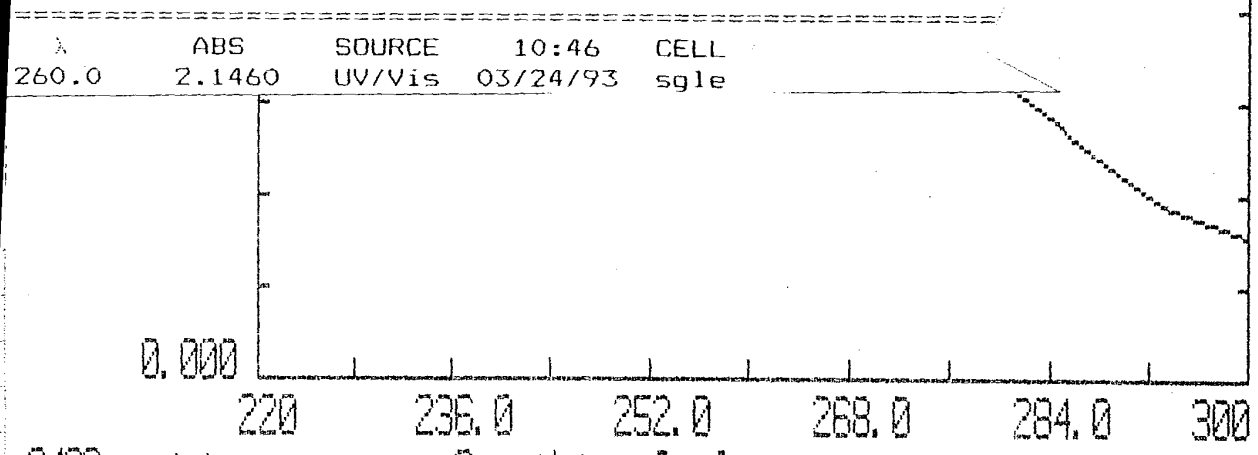
1018

AR93

DUAL λ 01 Abs Factor = 1.000
 Read average = 01
 S# 260.0 280.0 $\lambda A/\lambda B$ S# 260.0 280.0 $\lambda A/\lambda B$

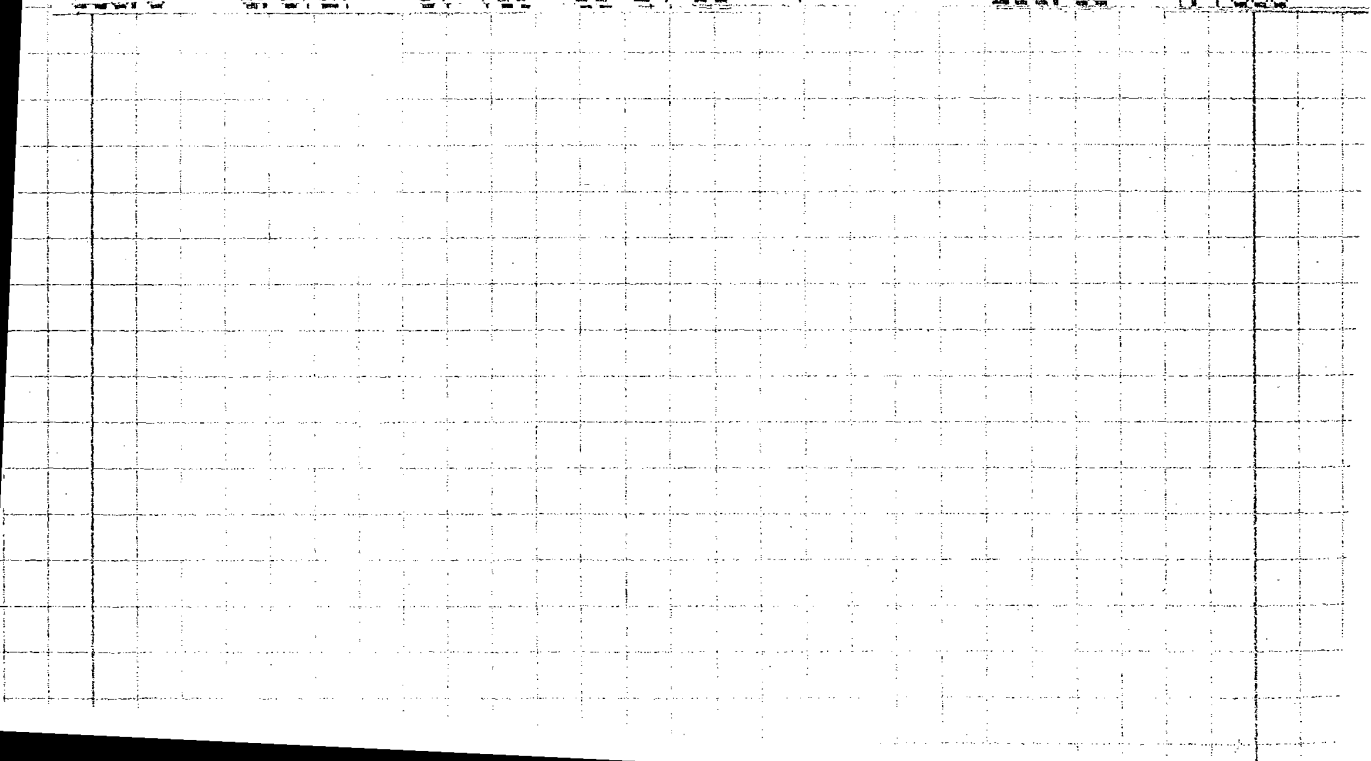
1	1.7823	1.0880	1.638	VEESP4 CB,GC
2	0.9070	0.5423	1.672	VEESP7 CB,GC
3	0.3790	0.2806	1.350	} Cathay's Omega Megaprep
4	0.4608	0.3403	1.354	
5	0.4343	0.3387	1.282	

#1 $89.115 \mu\text{g/ml} = 0.089115 \mu\text{g/ml} = 4.456 \mu\text{g/50 ml}$
 #2 $45.35 \mu\text{g/ml} = 0.04535 \mu\text{g/ml} = 2.27 \mu\text{g/50 ml}$



λ ABS SOURCE 10:49 CELL
 300.0 -0.3727 UV/Vis 03/24/93 1

TRACE
 λ ABS
 260.00 1.7668



12-well plates.

125

Have VEE SP3: PP#2

" 2D

VEE SP2: #2D

" 4D

VEE SP4: PP#4

V-1-2: PP#1

" PP#2

" PP#4

C-29-2-2D

" 4D

" PP#6

All the above samples were PCR + (ie appropriate length bp)
- Also have 3 blue colonies on Plaque assays set up
on _____

- Have plaques to pick from 18-Mar-93 Plaque assay

Colonies	1	2	3	4	5	
C-29-2 2D	ND	ND	ND	ND	ND	(+) = P _i present
C-29-2 4D	ND	ND	ND	ND	ND	(-) = no plaques
V-1-2 PP#1	1(-)	2(-)	3(-)	4(-)	5(-)	
2	6(-)	7(-)	8(-)	9(-)	10(-)	
3	11(-)	ND	ND	ND	ND	
4	12(-)	13+	14+	15(-)	16(-)	
many PH SINI	20	17 Blue	18+	ND	ND	
1 PH	4D	19+	20+	21(-)	22+	23+ (many) Serial
PP6	24+	25+	26+	27+	28+	
VEE SP2 2D	29+	30+	31+	32+	33+	
4D	34+	35+	36+	37+	38+	
VEE SP3 -2D	39+	40+	41+	42+	43+	
4D	44+	45+	46+	47+	48+	
PP #1	49+	50+	51+	52+	53+	
2	54+	55+	56+	57+	58+	
3	59+	60+	61+	62+	63+	
4	64+	65+	66+	67+	68+	
many PH	VEE SP6 2D	ND	ND	ND	ND	
many PH	4D	ND	ND	ND	ND	
	PP1	69+	70+	71+	72+	
	2	73+	74+	75+	76+	
	3	77+	78+	79+	80+	
		81+	82+	83+	84+	

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384

1 APR 93

DNA	2C	V-1-2	SIN 1	VEESP2	VEESP3	VEESP6	PBBIII	Indinavir samples p 127
CTED	500	1,481	2,110	2,112	2,261	3,509	620	
GTH								
WARD PR	Pr#1							
VERSE PR	Pr#2							
DO	35.2	55						
TEMPLATE	0.5	5						
WARD PR	2.5	0.1						
VERSE PR	2.5	0.3						
RXN	5							
UFFER								
WTP	1 ut ea							
pool	0.3							
TV	50 ut							
mineral	50 ut							
oil								

45 ut of "Super" mix

Thermocycler Program:

Step cycle: 1 min @ 94°C
2 min @ 55°C
3 min @ 72°C } 30 cycles

Delay cycle: 7 min @ 72°C

Soak cycle: 4°C

Super mix

10x RXN buffer	5 x	61 =
dATP	1 x	61
dCTP	1 x	61
dGTP	1 x	61
dTTP	1 x	61
HIS F1	0.4 x	61
HIS R1	0.3 x	61
H2O	3.5 x	61
Tag pool	0.3 x	61
		61/2

at Have 24 x 4 plaque picks - $48 \div 12 = 4$ - 12 well plates ^{actually ended up w 90%}
 → Have 11 samples that are frozen @ -70 (see list top p126)
 → Have 3 blue plaques from Plaque assay of —
 These plaques have some fungus around edge of plates.
 use separate 12-wells for each of these plaque picks.
 Set up 8-12 well plates w/ 5×10^5 cells/well. Add
 2 ml of media/well.

Will have 96⁹⁷ wells x 5×10^5 cells/well = 48.5×10^6 cells total
 Have 1.41×10^6 cells/ml (97.1% viable)
 34.4 ml of cells + 159.6 ml of TNM-FH (complete)

PCR analysis of Recombinant Virus

- Have 85 samples. Media clarified by centrifugation at ~~3,800~~^{3,000} rpm for 3 min in 1.5 ml tubes. Cells discarded. Media transferred to 4-1.5 ml Nalgene tubes and frozen @ -70°C
- Transfer 750 μ l of supernatant to a 1.5 ml Eppendorf tube and add 750 μ l of a solution of 20% PEG in 1M NaCl. ~~Have~~ Have 85 samples = 63.75 ml of PEG/NaCl solution.

Make up 75 ml: 15g PEG
 18.75 ml of 4M NaCl
 QS to 75 ml w/ H₂O

Have following samples:

V-1-2 PP1- (1-5)
 " 2- (1-5)
 " 3- (1)
 " 4- (1-5)

SIN 1 2D 1-2

" 4D R 5

" PP6 1-5

VEE SP2 2D 1-5

VEE SP2 4D 1-5

VEE SP3 2D 1-5

VEE SP3 4D 1

VEE SP3 - PP1-1;2

PP2-1,2

PP3-1,2

PP4-1,2

VEE SP6 PP1-1;2

PP2-1,2

5

5

1

5

2

5

5

5

5

5

5

1

2

2

2

2

2

2

2

#15

1-16

#3

17, 18

#5 19-23

#5 24-28

#5 29-33

#5 34-38

#5 39-43

#5 44-48

#5 49-53

#5 54-58

#5 59-63

#5 64-68

#5 69-73

#5 74-78

#5 79-83

#5 84-88

used red #15

not including #25

48

59

69

79

89

99

109

119

129

139

149

159

169

1st 9(+)

PP1- 1 2 3 4 5 PP2- 1 2 3 4 5 PP3/PP4 1 2 3 4 5 2D

14816P
SIAM = 2/106P

4072
3054
2036
1636

2036
1636

1018

BOTTOM
18-36

2	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2
2D	40	PP6	2D	40
SIAM	SIAM	VEESPZ	VEESPZ	VEESPZ

2 APR 93 93

VEESP2	VEESP3	VEESP4	VEESP5	PP2	PP3	PP4	VEESP6
4D	2D	4D	4D	1 2	1 2	1 2	1 2
1 2 3 4 5	1 2 3 4 5	1 2	1 2	1 2	1 2	1 2	1 2

#37-86

PP1- 1 2 3 4 5 PP2- 1 2 3 4 5 PP3/PP4 1 2 3 4 5 2D

Also same size as 1st

2 APR 93

Have additional blue plaques to pick from 18 Mar 93 @ Plaque assay

C-29-2 2D ^{# of plaques}
 VEESp6 2D 16
 VEESp3 4D 46
 pBBIII 83
 67

Will setup 6 plates, Will need 0.5×10^6 cells/well = 36×10^6 cell total.

Have 2 flasks: (ea w/ 75 ml cells)
 0.64×10^6 ← used this flask
 0.58×10^6
 0.61×10^6

50 ml cells + 88 ml TNM F.H (complete)

93 C-29-2 2D = 87
 VEESp6 2D = ~~88-95~~ 104-88 - ~~104~~ 103 ⁸⁷
 VEESp3 4D ~~95-104~~ 105 - ~~104~~ 149 ¹⁰⁴
 pBBIII ~~905-107~~ 152-154 ¹⁰⁵
 150-152 ⁴⁶
 151

	#		
1	C-29-2 2D	87	pp#9 VEESp3 4D 115
1	VEESp6 2D	88	10 114
2		89	11 115
3		90	12 116
4		91	13 117
5		92	14 118
6		93	15 119
7		94	16 120
8		95	17 121
9		96	18 122
10		97	19 123
11		98	20 124
12		99	21 125
13		100	22 126
14		101	23 127
15		102	24 128
16		103	25 129
1	VEESp3 4D	105	26 130
2		106	27 131
3		107	28 132
4		108	29 133
5		109	30 134

ME93 12-well plates (Cont.)

	#
34	VEESP 3 4D 138
35	139
36	140
37	141
38	142
39	143
40	144
41	145
42	146
43	147
44	148
45	149
~~46~~	pBBIII 150
	151
	152

Have additional blue plaques to pick from 18 Mar 93 Plaque Assay
 Done $\frac{442}{5} \cdot 1.5 \times 10^4 = 11.32 \times 10^6$ cells/ml.

C-29-2 2D 16

VEESP6 2D ~~46~~ 46

PBBIII ~~5~~ 5

6.1

Will set up 6 plates. Will need 0.5×10^6 cells/well = 36×10^6 cells total.

27.3 ml of cells + 116.7 ml of TMM FH (complete)

93	C-29-2 2D	153	Polyhedra observed	VEESP6 2D	187	+	~~46~~ 46
		154	+		188	+	
		155	+		189	+	
		156	+		190	+	
		157	+		191	+	
		158	+		192	+ few	
		159	+		193	+	
		160	+		194	+	
		161	+		195	+	
		162	+		196	+	
		163	+		197	+	
		164	+		198	+	
		165	+		199	+ few	
		166	+		200	+	
		167	+ few		201	+	
		168	+		202	+	
	VEESP6-2D	169	+		203	+	
		170	+		204	+ few	
		171	+		205	+	
		172	+		206	+	
		173	+		207	+ few	
		174	+		208	+	
		175	+		209	+	
		176	+		210	+	
		177	+		211	+	
		178	+		212	+	
		179	+		213	+	
		180	+		214	+	
		181	+		PBBIII ~~215~~	-	
		182	+		216	-	
		183	+		PBBIII 217	-	

Will need 0.5×10^6 cells/slide.

Have 2 Sf9 preps

see this
flask

→ #1 $\frac{548}{5} \cdot 1.5 \cdot 10^4 = 1.644 \times 10^6$ cells/ml (Have dead cell)

#2 $\frac{650}{5} \cdot 1.5 \times 10^4 = 1.95 \times 10^6$ cells/ml (few large clump)

Have 57 samples; will need 35 ^{wells} ~~spaces~~ for VEE = 5 slide

10 wells for CCHF = 2 slide

12 wells for SIV = 2 slide

Make up 10 slides = ~~80 wells~~ 10 x 3.2 = 32 ml of media
and containing 5×10^6 cells = 3 ml of cells + 29 ml of media.

Media = TNM FH

+ 10% FBS

+ Gent

+ Penicillin

136

SINGLE λ 01 Abs Read average = 01
 S# 600.0 S# 600.0 S# 600.0

1 -0.0030
 2 0.3186 ~~0.3186~~ TOP10F' — Started culture
 at 10:15A

λ ABS SOURCE 12:03 CELL
 600.0 -0.0030 Visible 03/25/93 1

SINGLE λ 01 Abs Read average = 01
 S# 600.0 S# 600.0 S# 600.0

3 0.0000
 4 0.5745 TOP10F' cell

λ ABS SOURCE 12:44 CELL
 600.0 -0.0701 Visible 03/25/93 1

at 37°C with shaking at 200 rpm.

93 Use SOP: Plasmid Max Prep Method 2: T₁ to Rotor
method (Ref: Sanger et al Biochem + Biophysical Res Comm
111: 835-842) to isolate plasmid DNA.

Quantitation at $A_{260/280}$ w/ scans are on p 146 this notebook.

Summary of PCR results - (Positive results only)

			PP	EXPECTED LENGTH	LENGTH	RESULT
10 MAR 93	VEE SP3		2	2,267	2,267	-
	VEE SP6		1	3,059	2,300	-
	V-1-2		1	1,481	1,481	-
	"		2	1,481	1,481	-
12 MAR 93	C-29-2		6	2,300	2,300	-
	VEE SP6		4	3,059	2,300	-
18 MAR 93	VEE SP2 2D			2,112	2,112	-
	" 4D			2,112	2,112	-
	VEE SP3 2D			2,267	2,112	-
	VEE SP3		1	2,267	1,600	-
			2	2,267	2,267	-
	V-1-2		1	1,481	1,481	-
			2	1,481	1,481	-
			4	1,481	1,481	-
	C-29-2 2D			2,300	2,300	-
	V-1-2 PP1 -		1	1,481	1,481	-
2 APR 93			2	1,481	1,481	-
			3	1,481	1,481	-
			4	1,481	1,481	-
			5	1,481	1,481	-
	PP2 -		1	1,481	1,481	-
			2	1,481	1,481	+
			3	1,481	1,481	-
			4	1,481	1,481	-
	VEE SP2 4D PP5			2,112	2,112	+
	~~PP2~~					
	VEE SP6 PP1 - 2			3059	~2,100	-
	PP3 - 1			3059	~2300	-

FA

Will need 0.5×10^6 cells/slide

Have 2.559 cell preps.

#1 $\frac{5.48}{5} \cdot 1.5 \times 10^4 = 1.644 \times 10^6$ cells/ml (Have dead cell)#2 $\frac{6.50}{5} \cdot 1.5 \times 10^4 = 1.95 \times 10^6$ cells/ml (few large clumps)

Set up 10 slides:

 10×3.2 ml/slide = 32 ml of INM. FH (complete)containing 5×10^6 cells total. = 3 ml of cells + 29 ml of media.ALL AB = 1:100
IN 20% FBS/PBS

1H = 1st AB

1H = 2nd AB

Observation
of PH (2 APP)

PCR

PH FA # CELLS

Well #

1 C-29-2 2D 25 FEB 93

2 P-2 C-29-2 Day 2 3/12/93 3/17/93

3 " " Day 4 " "

4 C-29-2 PP#1 27 Mar 93

5 C-29-2 2D #153

6 " " #154

7 " " #155

8 Uninfected

1 C-29-2 2D #156

2 " " #157

3 " " #158

4 " " #167

5 C-29-2 #6

6 Well not used

7 " "

8 Uninfected

1 P-2 Sin 1 Day 2 3/22/93 3/17/93

2 " " Day 4 " "

3 " " Day 6 " "

4 Sin 1 #6

5 Sin # 1-2D PP1 26 Mar 93

6 " " PP2 "

7 " " 4D PP1 "

8 Uninfected

1 Sin 1-4D PP2 26 Mar 93

2 " " PP3 "

3 " " PP4 "

4 " " PP5 "

5 " " PP6-1 "

6 " " b-2 "

7 " " b-3 "

1	2	3	4	5
5	6	7	8	9

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FA (cont.)

Will#	Observation of PH (2AOR)	PCR	FA
CLONE 1 P-2 V-1-2 #1 3/12/93 3/17/93	-	-	4-+4+
CLONE 2 " " #2 " "	-	-	-+3+
3 " " #3 " "	-	-	---
4 " " #4 " "	-	-	---
CLONE 5 V-1-2 PP1- 8 Mar 93 UNBELIEVABLE	-	+	-+4+
CLONE 6 " PP2 " (vii)	-	+	*-+4+
7 " PP4 "	-	-	- - -
8 Uninfected	-	-	- - -
1 VEE SP2 Day 2 3/12/93 3/17/93	-	+	- - -
CLONE 2 " Day 4 " WOW	+	+	* ⊖ +5+
3 VEE SP2 20 25 Feb 93	-	+	- - -
4 " 27 Feb 93 Media	-	-	- - -
5 VEE SP2 #1 8 Mar 93	-	-	- - -
6 " #2 "	-	-	- - -
7 " #3 "	-	-	- - -
8 Uninfected	-	-	- - -
1 VEE SP2 #4	-	-	- - -
2 " #5	-	-	? ? 1.
3 " #4 8 Mar 93	-	-	- - -
4 VEE SP3 20 25 Feb 93	-	+	- - -
5 VEE SP3 1 8 Mar 93	-	-	- - -
6 " 2 " (maybe another PP#1)	-	+	- - -
CLONE OUT VEE SP3 PP#1 27 Mar 93	+	50/50 FA/PH	+ + 4
8 Uninfected	-	-	- - -
CLONE 1 VEE SP3 4D PP#2 27 Mar 93	+	60/40 + maybe later	+ + 4
CLONE 2 " " PP#3 "	+	30/20	+ + 4
CLONE 3 " " PP#4 "	+	50/50	+ + 4
CLONE 4 " " PP#5 ✓ "	+	(Best safety) 80/20	+ + 4
CLONE 5 " " PP#6 "	+	50/50	+ + 3+
CLONE 6 " " PP#7 ✓ "	+	70/30	* + + 4+
7 VEE SP6 4	-	+/- band missing	- - -
8 Uninfected	-	-	- - -
1 VEE SP6 1 8 Mar 93	-	+/- band missing	+ ? 1+
2 " 4 "	-	-	- - -
CLONE 3 VEE SP6 20 PP#1 27 Mar 93	+	+/- band missing	+ + 4+
4 " " PP#2 "	+	-	+ + 2+
CLONE 5 " " PP#3 "	+	-	* + + 4+
6 " " PP#4 "	+	+/- band missing	+ + 2+
7 " " PP#5 "	+	-	+ + 2+

A) cont.

145

cell #				Observation of PH	PCR	FA
1	VEE SP6	2D	PP#6 27 Mar 93	~~Observation of PH (PP#6) +~~	~~MAA~~	~~MAA~~ ++ 2+
2	"	"	PP#7 "	+		* ++ 4+
3	VEE SP6 - 2D		169	+	ND	++ 2+
4	"		170	+	ND	++ 4+
5	"		171	+	ND	++ 1+
6	"		192	+	ND	++ 2+
7	"		199	+	ND	(2+) ++ 2+
8	uninfected			-		-

#2 looks better than #4

only a few PH+

different pattern

DUAL λ 01 Abs Factor= 1.000

Read average = 01

S# 260.0 280.0 $\lambda A/\lambda B$ S# 260.0 280.0 $\lambda A/\lambda B$ 5 ut in 500 ut H₂O = 1:101 dil Maxiprep

11 -0.0001 -0.0001 1.000

12 0.1219 0.0817 1.491 pBBIII - 500 ut of sample

13 0.1107 0.0654 1.692 VEE SP 1-1 - 500 ut of sample

14 0.0583 0.0345 1.689 VEE SP 5-10 250 ut of sample

15 0.0679 0.0457 1.484 VEE SP 5-11-1 500 ut of sample

#12 615.595 $\mu\text{g/ml}$ = 0.615595 $\mu\text{g/ut}$ = 307.8 μg #13 559.035 $\mu\text{g/ml}$ = 0.559035 $\mu\text{g/ut}$ = 279.5 μg #14 294.415 $\mu\text{g/ml}$ = 0.294415 $\mu\text{g/ut}$ = 13.6 μg #15 342.895 $\mu\text{g/ml}$ = 0.342895 $\mu\text{g/ut}$ = 171.4 μg

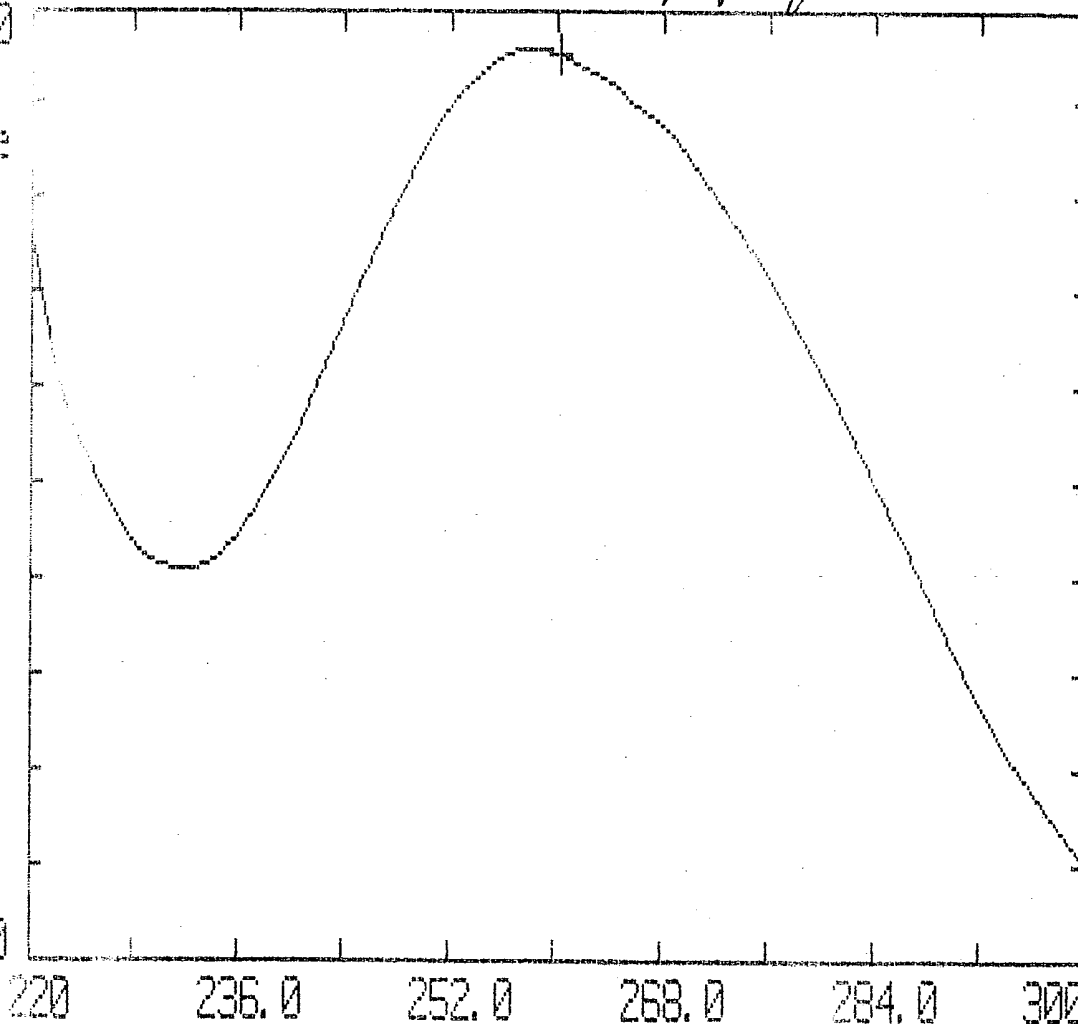
λ	ABS	SOURCE	07:41	CELL
260.0	0.0000	UV/Vis	03/31/93	1

Scan # 01.02 pBBIII - Maxiprep of 30MAR93

0.130

Display Mode
[Single]Function
[Abs]

0.000



2400 nm/min

Smoothing [no] pts.

TRACE

DUAL λ 01 Abs Factor=1.000

Read average = 01

S# 260.0 280.0 $\lambda A/\lambda B$ S# 260.0 280.0 $\lambda A/\lambda B$

50ul sample: EtOH centrifuged for 30 min -

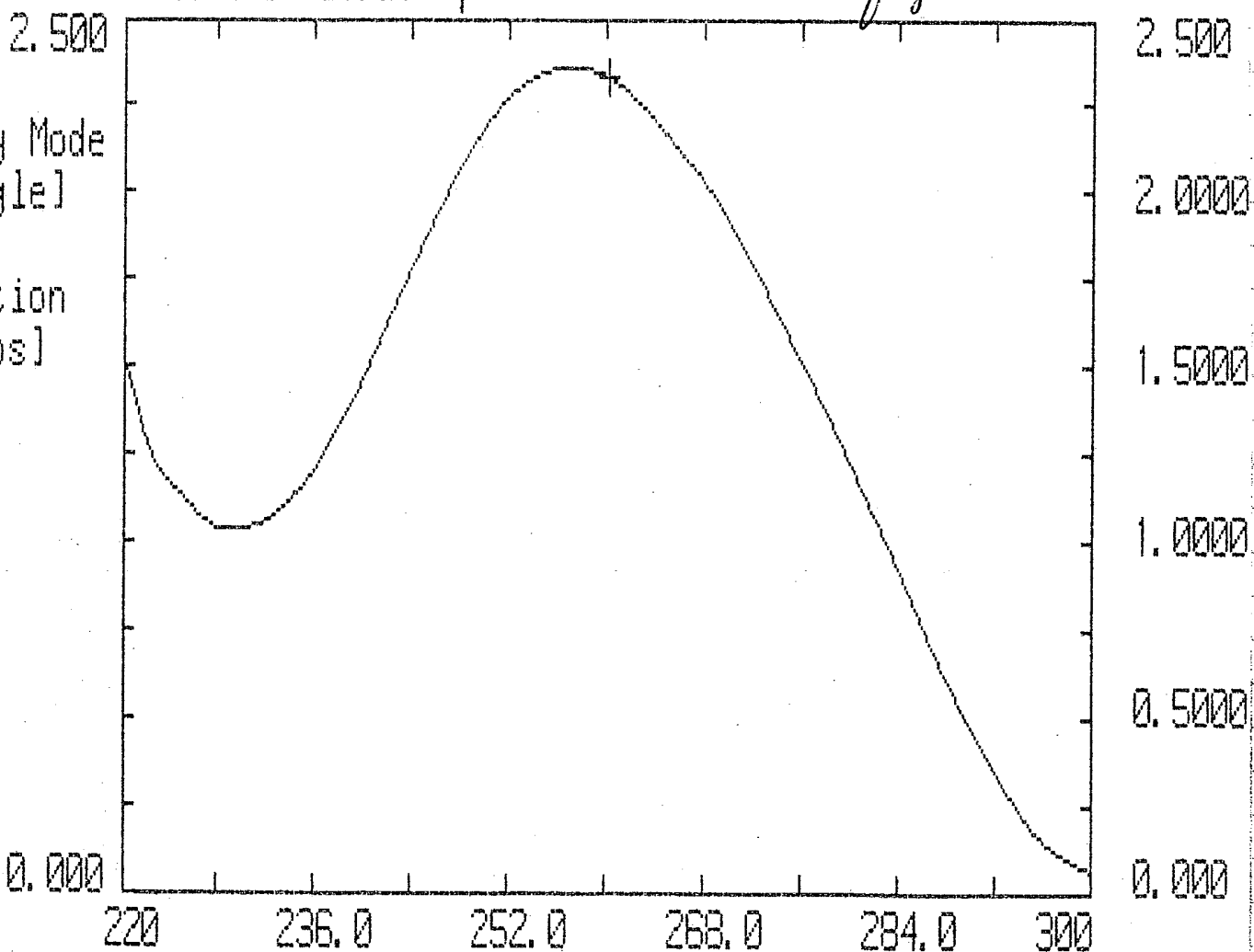
1	2.3634	1.3080	1.806	pBBIII
2	1.5169	0.8047	1.885	VEES P1-1
3	1.0523	0.5585	1.884	VEES P5-10
4	0.5744	0.3090	1.858	VEES P5-11-1

#1 $8.118.17 \mu g/ml = 0.11817 \mu g/ml = 5.91 \mu g$ in sample

PR93
(1-2)
(3-9)
(-12)
(-16)
(-18)
(-23)
(-25)
(-26)
[scribbles]

λ 260.0 ABS 0.5755 SOURCE UV/Vis 15:57 CELL 03/31/93 sqle

Scan # 01.01 pBBIII - 2nd centrifugation



Smoothing [no] pts.

TRACE

CO BRL

LIFE TECHNOLOGIES, INC.
GAITHERSBURG, MD 20884-9980 U.S.A.SA
No. **CMU103**
H I5'GATC C-3'
3'CTAG G-5'units; 10 U/ μ l
at -20°C (not frost-free)

ption:
I I is isolated from *Bacillus amyloliquefaciens* H. One unit is the
nt of enzyme required to cleave 1 μ g of lambda DNA in one hour at
in the appropriate buffer.

ponents:SA *Bam*H I
B4 REAct® 3Lot No. **CMU103**
Lot No. **CME102**

ge Buffer:
M Tris-HCl (pH 7.5)
1 DTT
mM Na₂EGTA
mM KCl
g/ml BSA
(v/v) glycerol
(w/v) Triton X-100

Reaction Conditions:

Use REAct® 3. REAct® 3 is the
10X concentrate assay buffer
supplied for use with *Bam*H I.
Dilute 1 part in 10 in final
reaction mixture.

Final concentration:

50 mM Tris-HCl (pH 8.0)
10 mM MgCl₂
100 mM NaCl

Assay at 37°C.

Store buffer at +4°C or -20°C.

lity Control Data: See Back Page

on® is a registered trademark of Rohm & Haas, Philadelphia, PA.

Effective: 02/07/92

BRL Bethesda Research Laboratories
Life Technologies, Inc.
5207SH

Gaithersburg, MD 20877 USA

Lot # **9MB108**

5'-AAGCT T-3'

Hind III

3'-T TCGA1A-5'

10,000 units; 50 U/ μ l
Store at -20°C (not frost-free)

Hind III is isolated from *Haemophilus influenzae* Rd.
One unit is the amount of enzyme required to cleave
1 μ g of lambda DNA in one hour at 37°C
in the appropriate buffer.

Storage Buffer:

10 mM Tris-HCl
(pH 7.4)
200 mM NaCl
0.5 mM Na₂EDTA
1 mM DTT
500 μ g/ml BSA
50% (v/v) glycerol

Reaction Conditions:

Use REAct® 2. REAct® 2
is the 10X concentrate assay
buffer supplied for use with
Hind III. Dilute 1 part in
10 in final reaction mixture.
Final concentration:
50 mM Tris-HCl (pH 8.0)
10 mM MgCl₂
50 mM NaCl
Assay at 37°C.
Store buffer at +4°C
or -20°C.

Quality Control Data:

Endonuclease Assay: Cleavage only at recognition site
at 100-fold excess digest of λ DNA.

Exonuclease Assay: Using 1 pmol radiolabeled termini
in a 50 μ l reaction and up to 20 units of enzyme for
one hour, the following results were observed:
1 unit of enzyme removed $\leq$ 0.3% label from 5' ends.
1 unit of enzyme removed $\leq$ 0.3% label from 3' ends.

Ligation/Recut Assay: On λ DNA: $\geq$ 95% ligation in
one hour at 22°C. 100% recut.
REFER TO THE BRL CATALOGUE AND REFERENCE GUIDE FOR
NOTES ON CONDITIONS WHICH AFFECT ENZYME ACTIVITY.

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC
OR THERAPEUTIC USE IN HUMANS OR ANIMALS.

RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE IN HUMANS OR ANIMALS.

Printed on Recycled Paper

restriction endonuclease digestion of λ EE SP7 and
 SP 4 PCR products (p 122-123) ~~and~~

1PR93

#1-2)

3-9)

10-12)

13-16)

17-18)

19-23)

24-28)

29-36)

37-40)

41-44)

45-48)

49-52)

restriction endonuclease digestion of pBBTII.
 pBBTII (Amritogen Col*)) 10 μ g in $\frac{100}{100}$ μ l

Added 0.1 μ l of 5M NaCl to PCR product digestion tubes
 and also pBBTII +.

26 colonies = 52 ml + 1 septa = 54 ml = 135 ul of AM

DUAL	260.0	280.0	AA/AB	S#	Abs	Factor= 1.000	Read average = 01
	260.0	280.0	AA/AB	S#	260.0	280.0	AA/AB

50ul sample

1 0.8666 0.4047 2.141 VEE SP 4/H/B/GB/GC - 2APR93

2 1.0094 0.5840 1.728 VEE SP 7/H/B/GB/GC - "

#1 43.33ug/ml = 0.0433ug/ul = 2.17ug total sample

#2 50.47ug/ml = 0.05047ug/ul = 2.5235ug total sample

ABS	SOURCE	09:18	CELL
260.0	1.0318 UV/Vis	04/05/93	sgle

DUAL	260.0	280.0	AA/AB	S#	Abs	Factor= 1.000	Read average = 01
	260.0	280.0	AA/AB	S#	260.0	280.0	AA/AB

1 0.0051 0.0057 .9000 PBB II / B/H/CIP

2 2.7561 1.8150 1.518

#2 137.8ug/ml = 0.1378ug/ul = 6.89ug in total sample.

GIBCO BRL

LIFE TECHNOLOGIES, INC.
GAITHERSBURG, MARYLAND 20898

5224SC

Lot # BFT114

T4 DNA Ligase

500 units; 1 U/ul
Store at -20°C (not frost-free)

Description:

T4 DNA Ligase can be used to join DNA fragments with staggered or blunt ends and to repair nicks in double-stranded DNA having 3'-hydroxyl and 5'-phosphate ends. The enzyme is isolated from *E. coli* lambda lysogen NM989.

Unit Definition:

One (Weiss) unit catalyzes the exchange of 1 nmol ³²PPi into [γ-³²P]ATP in 20 minutes at 37°C (1).

Storage Buffer:

10 mM Tris-HCl
(pH 7.5)
50 mM KCl
1 mM DTT
50% (v/v) glycerol

5X DNA Ligase Reaction Buffer:

250 mM Tris-HCl (pH 7.6)
50 mM MgCl₂
5 mM ATP
5 mM DTT
25% (w/v) polyethylene glycol-800
Store buffer at -20°C.
Due to the presence of dilute ATP, the shelf life of the buffer is 6 months.

NOTE:

FOR OPTIMAL TRANSFORMATION, DILUTE THE LIGATION REACTION >5-FOLD PRIOR TO ADDING TO COMPETENT CELLS (2).

Quality Control Assays:

Ligation of pBB and VEESP4, ~~VEESP7~~

H ₂ O	5X ligase buffer	pBB ^{III} (0.25 µg)	VEESP4	VEESP7	T4 DNA LIGASE	TV(µl)	# of COLONIES 8 APR 93
1.8	6	1.8	17.4 (0.75 µg)	—	3	25.2 30	2 (#1-2)
6.3	4	"	5.9 (0.25)	—	2	13.7 20	7 (#3-9)
10.4	4	"	1.8 (0.08)	—	2	9.6 20	3 (#10-12)
10.97	4	0.73	2.3 (0.1)	—	2	9.03 20	4 (#13-16)
4.3	6	1.8	—	14.9 (0.75)	3	25.7 30	2 (#17-18)
7.2	4	"	—	5.0 (0.25)	2	12.8 20	5 (#19-23)
10.6	4	"	—	1.6 (0.08)	2	9.4 20	2 (#24-25)
18.27	6	0.73	—	2.0 (0.1)	3	11.73 30	1 (#26)
12.2	4	1.8	—	—	2	7.8 20	5 ~~ctrl~~
12.2	4	1.8 pBB ~~mutant~~	—	—	2	20	TNTC (~~ctrl~~)
12.2	4	1.8 pBB 0/HKIP	—	—	2	20	5 ~~ctrl~~
Inc	20						

SINGLE λ 01 Abs
 S# 600.0 S# 600.0 Read average = 01
 S# 600.0
 1 0.0000
 2 0.7026 *top 10 F' cells*

=====

λ	ABS	SOURCE	09:15	ST	CELL
600.0	0.0000	Visible	04/07/93	1	

=====

DUAL λ 01 Abs Factor = 1.000
 Read average = 01
 S# 260.0 280.0 $\lambda A/\lambda B$ S# 260.0 280.0 $\lambda A/\lambda B$

Scut sample

S#	260.0	280.0	$\lambda A/\lambda B$	S#	260.0	280.0	$\lambda A/\lambda B$
1	2.8474	1.8137	1.570	<i>Menigex</i>	3	VEE SP 4 2-3	
2	2.8106	1.7807	1.578	"	9	VEE SP 4 2-9	
3	2.9440	2.0774	1.417	"	17	VEE SP 7 5-17	
4	2.0909	1.1685	1.789	"	26	VEE SP 7 8-26	

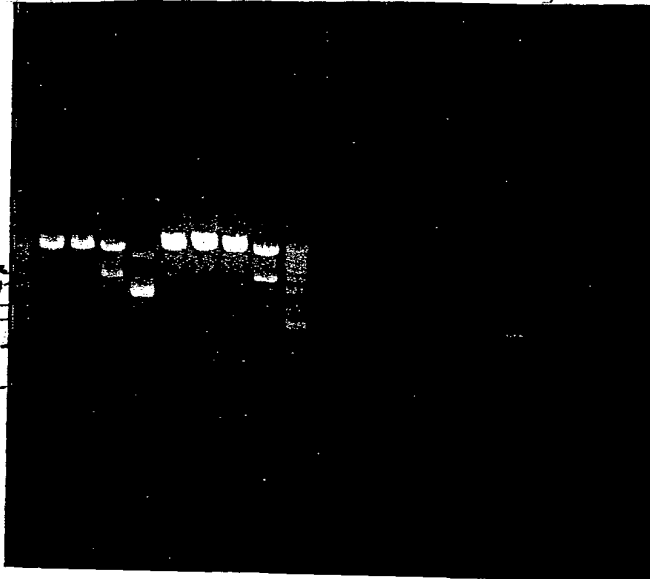
#1 0.14237 $\mu g/\mu l$ = 1.2 μg total sample
 #2 0.141 $\mu g/\mu l$ = 1.0 μg total sample
 #3 0.147 $\mu g/\mu l$ = 1.36 μg total sample
 #4 0.105 $\mu g/\mu l$ = 5.23 μg total sample

=====

λ	ABS	SOURCE	14:05	CELL
260.0	2.0902	UV/Vis	04/19/93	sgle

=====

4072
 3084
 2086
 1636
 1018
 507



-1018
 -507

4072
 3084
 2086
 1636
 1018
 507



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